BEST AVAILABLE COPY

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 July 2004 (22.07.2004)

PCT

(10) International Publication Number
WO 2004/061410 A2

(51) International Patent Classification⁷: G01N

(21) International Application Number: PCT/US2003/037090

(22) International Filing Date: 16 December 2003 (16.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/434,075 18 December 2002 (18.12.2002) US

(71) Applicants (*for all designated States except US*): CIPHERGEN BIOSYSTEMS, INC. [US/US]; 6611 Dumbarton Circle, Freemont, CA 94555 (US). QUEEN ELIZABETH HOSPITAL [CN/CN]; 30 Gascoigne Road, Kowloon, Hong Kong SAR (CN).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): YIP, Timothy, Tak, Chun [CN/CN]; 1, Kapok Path, Westwood, Palm Springs, Yuen Long N.T., Hong Kong SAR (CN). CHO, Chi, Shing [/CN]; Flat 9B, Block 4, The Tolo Place, Sunshine City, Ma On Shan, N.T., Hong Kong SAR (CN). AU, Siu, Kie [—/CN]; c/o Department of Clinical Oncology, Queen Elizabeth Hospital, 30 Gascoigne Road, Kowloon, Hong Kong SAR (CN). YIP, Tai-Tung [US/US]; 1532 Aster Court, Cupertino, CA 95014 (US). YIP, Christine, L. [US/US]; 1532 Aster Court, Cupertino, CA 95014 (US). YIP, Victor, F. [US/US]; 33008 Compton CT, Union City, CA 94587 (US).

(74) Agents: BENT, Stephen, A. et al.; Foley & Lardner, Washington Harbour, 3000 K Street, N.W. Suite 500, Washington, DC 20007-5101 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),

[Continued on next page]

(54) Title: SERUM BIOMARKERS IN LUNG CANCER

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
BM-1	2011	A	BM-37	3092	A	BM-73	64039	A	BM-109	2882	B
BM-2	2030	A	BM-38	3360	A	BM-74	66170	A	BM-110	2967	B
BM-3	2089	A	BM-39	3572	A	BM-75	74372	A	BM-111	2977	B
BM-4	2128	A	BM-40	3634	A	BM-76	75648	A	BM-112	2994	B
BM-5	2148	A	BM-41	4088	A	BM-77	77643	A	BM-113	3031	B
BM-6	2188	A	BM-42	4178	A	BM-78	78507	A	BM-114	3048	B
BM-7	2232	A	BM-43	4287	A	BM-79	83854	A	BM-115	3149	B
BM-8	2277	A	BM-44	4307	A	BM-80	101831	A	BM-116	3166	B
BM-9	2295	A	BM-45	4320	A	BM-81	104301	A	BM-117	3283	B
BM-10	2318	A	BM-46	4494	A	BM-82	125190	A	BM-118	3306	B
BM-11	2411	A	BM-47	4640	A	BM-83	132676	A	BM-119	3337	B
BM-12	2434	A	BM-48	4780	A	BM-84	148088	A	BM-120	3432	B
BM-13	2467	A	BM-49	5104	A	BM-85	2076	B	BM-121	3450	B
BM-14	2482	A	BM-50	6018	A	BM-86	2078	B	BM-122	3591	B
BM-15	2498	A	BM-51	6122	A	BM-87	2144	B	BM-123	3616	B
BM-16	2565	A	BM-52	6192	A	BM-88	2150	B	BM-124	3714	B
BM-17	2574	A	BM-53	6452	A	BM-89	2168	B	BM-125	3730	B
BM-18	2606	A	BM-54	6880	A	BM-90	2184	B	BM-126	3834	B
BM-19	2608	A	BM-55	7768	A	BM-91	2200	B	BM-127	3875	B
BM-20	2722	A	BM-56	8143	A	BM-92	2214	B	BM-128	3962	B
BM-21	2748	A	BM-57	8854	A	BM-93	2230	B	BM-129	3985	B
BM-22	2780	A	BM-58	8912	A	BM-94	2314	B	BM-130	3987	B
BM-23	2835	A	BM-59	9848	A	BM-95	2414	B	BM-131	4013	B
BM-24	2871	A	BM-60	10272	A	BM-96	2420	B	BM-132	4161	B
BM-25	2884	A	BM-61	11683	A	BM-97	2431	B	BM-133	4287	B
BM-26	3030	A	BM-62	13370	A	BM-98	2468	B	BM-134	4311	B
BM-27	3144	A	BM-63	14698	A	BM-99	2488	B	BM-135	4458	B
BM-28	3243	A	BM-64	16190	A	BM-100	2505	B	BM-136	4484	B
BM-29	3273	A	BM-65	16758	A	BM-101	2583	B	BM-137	4579	B
BM-30	3290	A	BM-66	19961	A	BM-102	2597	B	BM-138	4658	B
BM-31	3309	A	BM-67	25172	A	BM-103	2667	B	BM-139	4689	B
BM-32	3445	A	BM-68	18625	A	BM-104	2718	B	BM-140	4747	B
BM-33	3463	A	BM-69	23436	A	BM-105	2740	B	BM-141	4862	B
BM-34	3678	A	BM-70	30784	A	BM-106	2752	B	BM-142	4891	B
BM-35	3779	A	BM-71	41100	A	BM-107	2767	B	BM-143	5033	B
BM-36	3793	A	BM-72	46290	A	BM-108	2806	B	BM-144	5077	B

(57) Abstract: Certain biomarkers and biomarker combinations are useful in a qualifying lung cancer status in a subject. A diagnostic methodology employing these biomarkers and combinations can detect whether a subject has lung cancer.

WO 2004/061410 A2

European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

SERUM BIOMARKERS IN LUNG CANCER

BACKGROUND OF THE INVENTION

[0001] The present invention relates generally to the field of serum biomarkers in lung carcinoma. More particularly, the invention relates to serum biomarkers that can distinguish lung cancer from normal.

[0002] Lung cancer is the leading cause of cancer death worldwide, resulting in 150,000 deaths per year in the United States. The mortality rate from lung cancer is greater than the combined mortality from breast, prostate and colorectal cancers. On the basis of morphology, lung cancer can be broadly classified into four main categories namely, adenocarcinoma, squamous cell carcinoma, large cell undifferentiated carcinoma and small cell carcinoma. In Hong Kong from 1990 to 1996, the prpportions for adenocarcinoma, squamous cell carcinoma, large cell undifferentiated carcinoma and small cell carcinoma are 45.5%, 27.5%, 4.7% and 10.3% respectively. Both squamous cell carcinoma and small cell carcinoma are strongly associated with a smoking history.

[0003] Adenocarcinoma, squamous cell carcinoma, and large cell undifferentiated carcinoma are usually referred as "non-small cell carcinoma." They are relatively chemo-resistant, and hence the mainstay of treatment is surgery. By contrast, small cell carcinoma has a higher propensity for distant metastases and is mainly treated by chemotherapy.

[0004] Biopsy can be used to diagnose lung cancer, but it is an invasive procedure and, therefore, less than desirable. Other diagnostic methods for lung cancer include ultrasound and computed tomography (CT) scan.

[0005] It would be highly desirable to have a biomarker or combination of biomarkers capable of distinguishing between lung cancer and normal cells. In addition, a simple test could aid in tracking treatment progress and even identify molecular targets for therapy. The literature on lung cancer diagnosis has not disclosed heretofore such a biomarker or combination of biomarkers, however.

SUMMARY OF THE INVENTION

[0006] In accordance with the present invention, biomarkers and combinations of biomarkers are used to identify lung cancer. The method successfully distinguishes between lung cancer and normal states, and can be used to identify the particular type of lung cancer. In one embodiment, a method for qualifying lung carcinoma status in a subject (e.g., a patient) comprises analyzing a biological sample from the subject for one or more of the top 50 biomarkers as shown in Figure 2 or Figures 4A and 4B. Thus, to assess overall lung cancer risk versus normal, a biomarker is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-165, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268, or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310,

[0007] wherein the biomarker is differentially present in samples of a subject with lung cancer and a so-called "normal" subject that is free of lung cancer.

[0008] More preferably, one or more of the top 15 biomarkers as shown in Figure 2 or Figures 4A and 4B is used to qualify lung cancer status. Thus, for assessing overall lung cancer status versus normal, the protein is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-471, IM-510, IM-544, IM-474, and IM-155, or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70.

[0009] Still more preferably, one or more of the top 5 biomarkers as shown in Figure 2 or Figures 4A and 4B is used to qualify lung cancer status. In this instance, for overall lung cancer status versus normal, the biomarker is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, and IM-454, or

(B) WM-61, WM-447, WM-446, WM-133, and WM-119.

[0010] In one embodiment, the method measures a plurality of biomarkers. The plurality of biomarkers can be measured simultaneously.

[0011] Biomarkers that, by themselves, are able to identify lung cancer include the WM-446 and WM-447 protein biomarkers, and these are particularly preferred.

[0012] The present invention also provides a method for qualifying lung cancer status in a subject (e.g., a patient), comprising (A) providing a spectrum generated by subjecting a biological sample from said subject to mass spectroscopic analysis that includes profiling on a chemically-derivatized affinity surface, and (B) putting the spectrum through pattern-recognition analysis that is keyed to at least one peak selected from the top 50 biomarkers as shown in Figure 2 or Figures 4A and 4B. Thus, for qualifying overall lung cancer status, the biomarker is selected from the, group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-446, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268, or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-282, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

[0013] For assessing the overall lung cancer status, the pattern-recognition analysis may, for example, be paired to a pair of peaks selected from the group consisting of

(A) IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-266 and IM-522, IM- 266 and IM-544, IM-266 and IM-471, IM-474 and IM-151, IM-474 and IM-156, IM-474 and IM-544, IM-474 and IM-38, IM-522 and IM-507, IM-522 and IM-156, and IM-522 and IM-440;

or

(B) WM-447 and WM-59, WM-447 and WM-19, WM-447 and WM-118, WM-447 and WM-473, WM-19 and WM-59, WM-19 and WM-473, WM-19 and WM-369, WM-61 and WM-154, WM-61 and WM-369, WM-118 and WM-59 and WM-282 and WM-127.

[0014] More preferably, for assessing overall lung cancer status, the pattern-recognition analysis is keyed to a pair of peaks selected from the group consisting of

(A) IM-266 and IM-474, IM-266 and IM-544, and IM-156 and IM-522;

or

(B) WM-447 and WM-59, WM-447 and WM-19, and WM-19 and WM-59.

[0015] Alternatively, the pattern-recognition analysis for assessing overall lung cancer status may be keyed to a triplet of peaks selected from the group consisting of

(A) IM-266, IM-454 and IM-474; and IM-268, IM-474 and IM-544;

or

(B) WM-447, WM-19 and WM-473.

[0016] In other embodiments, the pattern-recognition analysis may be keyed to a combination of more than three peaks, more particularly to a combination of 4, 5 or 6 peaks, where the combination is selected from among the combinations shown in Tables 1 and 2 herein.

[0017] In each case, the biomarker is differentially present in samples of a subject with lung cancer and a normal subject.

[0018] The invention also contemplates a kit for detecting and diagnosing lung cancer, thereby to assess lung cancer status. Kits within the invention comprise, for example, (i) an adsorbent attached to a substrate that retains one or more of the biomarkers shown in Figure 2 or Figures 4A and 4B, and (ii) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent. An inventive kit may further comprise a washing solution and/or instructions for making a washing solution. The kits may include more than type of adsorbent, each present on a different substrate, *e.g.*, on a WCX and IMAC biochip. In addition, the kits may comprise one or more containers with biomarker samples, to be used as standard(s) for calibration. The substrate comprising the adsorbent may be designed to engage a probe interface and, hence, function as a probe in gas phase ion spectrometry, preferably mass spectrometry. Alternatively, the kit may further comprise a second substrate adapted to engage the probe interface, on which the substrate comprising the adsorbent is mounted.

[0019] The method and kit according to the invention produce an article of manufacture in which one or more biomarkers according to the invention are bound to an adsorbent, optionally contacted with a matrix or energy absorbing molecule.

[0020] The present invention also provides software for qualifying lung carcinoma status in a subject, comprising an algorithm for analyzing data extracted from a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, wherein said data relates to one or more biomarkers according to the invention. In one embodiment, the algorithm carries out a pattern-recognition analysis that is keyed to data relating to at least one of the biomarkers. In another embodiment, the algorithm comprises classification tree analysis that is keyed to data relating to at least one of the biomarkers. In yet another embodiment, the algorithm

comprises an artificial neural network analysis that is keyed to data relating to at least one of the biomarkers.

[0021] In certain embodiments, the present invention provides methods and kits that use serum amyloid a protein or a fragment thereof to qualify lung carcinoma status in a subject. In one of these embodiments, the serum amyloid a biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons. In another embodiment, the serum amyloid a biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons. In yet another embodiment, the serum amyloid a biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figures 1A-1D show all biomarkers identified with a Cu(II) IMAC3 ProteinChip® array format.

[0023] Figure 2 shows the top 50 biomarkers identified with a Cu(II) IMAC3 ProteinChip® array format.

[0024] Figures 3A-3O show all biomarkers identified with a WCX ProteinChip® array format.

[0025] Figures 4A and 4B show the top 50 biomarkers identified with a WCX ProteinChip® array format.

[0026] Figure 5 shows fragments of serum amyloid A (SAA) that are biomarkers according to the present invention.

[0027] Figure 6 shows identification of SAA biomarkers with an anti-SAA antibody.

[0028] Figures 7-16 are spectra from WCX chips in which all of the top 15 WCX marker peaks are labeled, along with various other peaks from among the top 50 WCX peaks. Red shows spectra from lung cancer patients and gray shows normals.

[0029] Figures 17-28 are spectra from IMAC chips in which all of the top 15 WCX marker peaks are labeled, along with various other peaks from among the top 50 IMAC peaks. Blue shows spectra from lung cancer patients and gray shows normals.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0030] In accordance with the present invention, a series of biomarkers associated with lung cancer has been discovered. In the present context, a biomarker is an organic biomolecule, particularly a polypeptide or protein, which is differentially present in a sample taken from a subject having lung cancer as compared to a comparable sample taken from a normal subject. A biomarker also may be differentially present in a sample taken from a subject with one type of lung cancer, *e.g.*, small cell carcinoma, as compared to a comparable sample taken from a subject with a different type of lung cancer, *e.g.*, adenocarcinoma or squamous cell carcinoma, or differentially present at different stages of a type of lung cancer. A biomarker is differentially present in samples taken from two groups of subjects if it is present at an elevated level or a decreased level in samples of the first group as compared to samples of the second group. More particularly, a biomarker is a polypeptide that is characterized by an apparent molecular weight, as determined by mass spectrometry, and that is present in samples from lung cancer subjects in an elevated or decreased level, as compared to subjects that do not have lung cancer. A biomarker is differentially present between two sets of samples if the amount of the biomarker in one sample set differs in a statistically significant way ($p < 0.01$) from the amount of biomarker in the other sample set.

[0031] The biomarkers of the invention can be used to assess lung cancer status in a subject. For example, they are capable of identifying lung cancer and successfully distinguishing it from normal subjects, thereby providing a way of diagnosing the presence or absence of lung cancer, including the presence or absence of a particular kind of lung cancer. In addition, the biomarkers are useful in assessing the risk of developing lung cancer, in staging of lung cancer and in assessing the effectiveness of treatment. Thus, "lung cancer status" in the context of the present invention includes, *inter alia*, the presence or absence of disease, the risk of developing disease, the stage of the disease, and the effectiveness of treatment of disease. Based on this status, further procedures may be indicated, including additional diagnostic tests or therapeutic procedures or regimens, such as endoscopy, biopsy, surgery, chemotherapy, immunotherapy, and radiation therapy.

[0032] In some instances, a single biomarker is capable of identifying lung cancer with a sensitivity or specificity of at least 85%, whereas, in other instances, a combination or plurality of biomarkers is used to obtain a sensitivity or specificity of at least 85%. The biomarkers and combinations of biomarkers thus can be used to qualify lung cancer status in a subject or patient.

[0033] The biomarkers according to the invention are present in serum. The biological sample used according to the present invention, however, need not be a serum sample. Thus, a biological sample for qualifying lung cancer status may be a serum, plasma or blood sample, although serum samples are preferred.

[0034] All of the biomarkers are characterized by molecular weight. A list of all the biomarkers obtained with the Cu(II) IMAC3 ProteinChip® array (Ciphergen Biosystems, Inc., Fremont, California, USA) is provided in Figures 1A-1D, and Figure 2 lists the top 50 biomarkers that distinguish between lung cancer and normal subjects that are identified by Cu(II) IMAC3 protocol described herein. Figures 3A-3O comprise a list of all the biomarkers obtained with the WCX2 ProteinChip® array, and Figures 4A and 4B comprise a ranking of the top 50 biomarkers that distinguish between (i) lung cancer and normal subjects, (ii) subjects with each of four types of lung cancer and normal subjects, and (iii) two types of lung cancer, *e.g.*, adenocarcinoma versus squamous cell carcinoma, as identified by WCX2 protocol described herein.

[0035] The top 50 biomarkers were determined by decision tree analysis using Biomarker Patterns™ software from Ciphergen Biosystems, Inc. Biomarkers other than those within the top 50 also are useful in distinguishing between subjects with lung cancer and normal subjects and may, in particular, appear in decision trees with multiple nodes. In preferred embodiments, one or more of the top 15 biomarkers are used, and in even more preferred embodiments, one or more of the top 5 biomarkers are used.

[0036] In each of Figures 1A-1D and 3A-3O, the number in the first column is the biomarker identifier. Thus, the first row in Figures 1A-1D relates to biomarker IM-1, the second row relates to biomarker IM-2, and so forth ("IM-" denoting biomarkers identified with the IMAC chip). Similarly, the first row in Figures 3A-3O relates to

biomarker WM-1 and the second row relates to biomarker WM-2 ("WM-" denoting biomarkers identified with the WCX2 chip). The number in the second column in Figures 1A-1D is the apparent molecular weight of the biomarker in daltons, as determined by mass spectrometry. In Figures 3A-3O, the apparent molecular weights for the biomarkers identified in the first column are reported in columns 3 through 11. The letter in the second column of Figures 1A-1D and the third column of Figures 3A-3O denotes the fraction in which the biomarker elutes in the protocol described herein; that is, biomarkers with an "A" elute in the first fraction, biomarkers with a "B" elute in the second fraction, and so forth. The fraction in which the biomarker elutes correlates with its pI, which biomarkers eluting at higher pH having a higher pI, and biomarkers eluting at lower pH having a lower pI.

[0037] Presenting the mass and affinity characteristics of a given biomarker within the invention, as in this description, characterizes that biomarker so as allow one to obtain and measure it, in accordance with the teachings herein. If desired, any of the biomarkers can be sequenced, in order to obtain an amino acid sequence, but this is not required to practice the present invention.

[0038] For example, a biomarker can be peptide mapped with a number of enzymes, such as trypsin and V8 protease, and the molecular weights of the digestion fragments can be used to search databases for sequences that match the molecular weights of the digestion fragments generated by the various enzymes. Alternatively, if the biomarkers are not proteins included in known databases, degenerate probes can be made based on the N-terminal amino acid sequence of the biomarker, which then are used to screen a genomic or cDNA library created from a sample from which the biomarker was initially detected. The positive clones can be identified, amplified, and their recombinant DNA sequences can be subcloned using techniques which are well known. Finally, protein biomarkers can be sequenced using protein ladder sequencing. Protein ladders can be generated by fragmenting the molecules and subjecting fragments to enzymatic digestion or other methods that sequentially remove a single amino acid from the end of the fragment. The ladder is then analyzed by mass spectrometry. The difference in masses of the ladder fragments identifies the amino acid removed from the end of the molecule.

[0039] Several biomarkers identified in accordance with the teachings of the present invention fit to serum amyloid A (SAA) or to a fragment of SAA. SAA is a well-known acute phase inflammatory marker. A number of the SAA biomarkers are identified in Figure 5 by both molecular mass and amino acid sequence. Most of these markers bound anti-SAA antibodies, as shown in Figure 6. The intact mass of SAA is 11.5 to 11.7 kD, and these biomarkers also have been identified by the present methodology.Fragments preferably have a molecular mass of at least about 200 Daltons, more preferably at least about 500 Daltons. In even more preferred embodiments, fragments have a molecular mass of at least about 800 Daltons, and most preferably at least about 1 Kilodalton.

[0040] In one embodiment, the fragments of SAA include a sequence of amino acids that is recognized by an epitope of an anti-SAA antibody. One way of identifying suitable fragments for use in the present invention is to enzymatically digest SAA and test the resulting fragments for the ability to bind to an anti-SAA antibody. Fragments that bind anti-SAA antibody can be sequenced using techniques well-known in the art, although the sequence of the fragment is not needed to practice the invention. In order to practice the invention with a fragment from the enzymatic digest that is identified as binding anti-SAA antibody, all that is required is to subject to the fragment to mass spectrometry to determine its mass.

[0041] The serum biomarkers according to the present invention were identified by comparing mass spectra of samples derived from sera from two groups of newly-diagnosed subjects, subjects with lung cancer and normal subjects. The subjects were diagnosed according to standard clinical criteria. Lung cancer subjects were histologically confirmed, and subjects without lung cancer were followed for at least 18 months following serum collection for any sign of lung cancer, to exclude subjects with asymptomatic lung cancer.

[0042] Sera from each group of subjects was collected, and fractionated with Q Ceramic HyperDF ion exchange resin (Biosepra SA, France) into six fractions which eluted at different pH. Fraction A comprised the flow through plus pH 9 eluant, Fraction B comprised the pH 7 eluant, Fraction C comprised the pH 5 eluant, Fraction D comprised the pH 4 eluant, Fraction E comprised the pH 3 eluant, and Fraction F

comprised isopropyl alcohol/acetonitrile TFA eluant. Fractions A through F are identified on Figures 7-28 as Fractions 1 through 6, respectively.

[0043] Each fraction was diluted and applied to a ProteinChip® array, either a Cu(II) IMAC3 or WCX2 chip array. Both of these chip arrays are produced by Ciphergen Biosystems, Inc. (Fremont, CA).

[0044] The Cu(II) IMAC3 is an "immobilized metal affinity-capture" chip, with a nitrilotriacetic acid surface for high-capacity copper binding and subsequent affinity capture of proteins with metal binding residues. Imidazole may be used in binding and washing solutions to moderate protein binding, including binding of non-specific proteins. Increasing the concentration of imidazole in the washing buffers reduces the binding of the target proteins. It is produced by photopolymerizing 5-methylacylamido-2-(N,N-biscarboxymethylamino)pentanoic acid (7.5 wt%) and N,N'-methylenebisacrylamide (0.4 wt%) using (-) riboflavin (0.02 wt%) as a photoinitiator. The monomer solution is deposited onto the chip substrate and irradiated to photopolymerize. The chip then is activated with Cu(II).

[0045] The WCX2 is a weak cation exchange array with a carboxylate surface to bind cationic proteins. The negatively charged carboxylate groups on the surface of the WCX2 chip interact with the positive charges exposed on the target proteins. The binding of the target proteins is reduced by increasing the concentration of salt or by increasing the pH of the washing buffers.

[0046] Following application of the eluant fraction, the chips were incubated to allow the polypeptides in the eluant to bind to the sites on the chip by an affinity interaction. After incubation, each chip array was washed to remove polypeptides that bind non-specifically and buffer contaminants. That chip then was dried, and an energy absorbing molecule or matrix was applied to it, to facilitate desorption and ionization in a mass spectrometer.

[0047] In the mass spectrometer, retained polypeptides were desorbed from the chip array by laser desorption and ionization in a ProteinChip® Reader, which is integrated with ProteinChip® Software and a personal computer to analyze proteins captured on chip arrays. The ion optic and laser optic technologies in the ProteinChip® Reader detects proteins ranging from small peptides of less than 1000 Da up to proteins of

300 kilodaltons or more, and calculates the mass based on time-of-flight. Ionized polypeptides were detected and their mass accurately determined by this Time-of-Flight (TOF) Mass Spectrometry.

[0048] The mass spectra obtained for each group were subjected to scatter plot analysis, to eliminate run-to-run variation. Protein clusters on the scatter plot that had the same pattern for both lung cancer and normal subjects, *i.e.*, protein clusters that were either elevated in both groups of subjects or depressed in both groups of subjects, were eliminated as potential biomarkers. The remaining polypeptides were further analyzed for their ability to accurately identify subjects with lung cancer. Because the molecular weights were derived from scatter plot analysis, and because of limits on the ability of mass spectrometry to resolve molecular weights, the "absolute" molecular weight values given in Figures 1A-1D and 3A-3O actually represent approximate molecular weights.

[0049] The biomarkers of this invention are characterized by their mass-to-charge ratio as determined by mass spectrometry. The mass-to-charge ratio of each biomarker is provided in Figures 1A-1D and 3A-3O. For example, IM-1 in Figure 1A has a measured mass-to-charge ratio of 2011. The mass-to-charge ratios were determined from mass spectra generated on a Ciphergen Biosystems, Inc. PBS II mass spectrometer. This instrument has a mass accuracy of about +/- 0.15 percent. Additionally, the instrument has a mass resolution of about 400 to 1000 m/dm, where m is mass and dm is the mass spectral peak width at 0.5 peak height. The mass-to-charge ratio of the biomarkers was determined using Biomarker Wizard^{TM} software (Ciphergen Biosystems). Biomarker Wizard assigns a mass-to-charge ratio to a biomarker by clustering the mass-to-charge ratios of the same peaks from all the spectra analyzed, as determined by the PBSII, taking the maximum and minimum mass-to-charge-ratio in the cluster, and dividing by two. Accordingly, the masses provided reflect these specifications.

[0050] The biomarkers of this invention are further characterized by the shape of their spectral peak in time-of-flight mass spectrometry. Mass spectra showing peaks representing the biomarkers are presented in Figures 7-28. The biomarker identifier numbers from Figures 2 and 4A-4B, respectively, are shown next to the peak, along

with their rank, which is indicated in parentheses below the biomarker identifier number.

[0051] The biomarkers of this invention are further characterized by their binding properties on chromatographic surfaces. Most of the biomarkers bind to IMAC (Cu) or WCX adsorbents (e.g., the Ciphergen® IMAC (Cu) or WCX ProteinChip® arrays) after washing as described herein.

[0052] Thus, a given molecular weight for a biomarker herein should be interpreted as the midpoint of a molecular-weight range. The accuracy of the mass spectrometer is +/- 0.15%, and the actual molecular weight for a biomarker is therefore the value given, +/- 0.15%. For example, the actual molecular weight for biomarker IM-273 is 11705 +/- 0.15%, or between 11687 and 11722. Often, the range surrounding the "absolute" value given in the figure is no more than +/- 5 daltons (2006 to 2016 for IM-1), generally no more than +/- 3 daltons (2008 to 2014 for IM-1), and often as small as +/- 1 dalton (2010 to 2012 daltons for IM-1).

[0053] CART® (Salford Systems, San Diego, CA), a classification and regression tree software, was used to determine whether a potential biomarker had predictive value in assessing lung cancer. A software macro randomly selected a subset of 15% of the peaks from Figures 1A-1D or Figures 3A-3O. The peaks and peak heights from each sample were provided to the CART® software for analysis. The software performed an iterative analysis until a single decision tree was generated that was capable of distinguishing between cancerous and non-cancerous. Each node in the resulting decision tree sorted based on the peak height of a single biomarker. A tree may contain any number of nodes, but generally contains from 1 to 6 nodes. From a practical standpoint in a commercial diagnostic test, a decision tree with fewer nodes is preferred. A total of 2000 decision trees, each based on a different 15% subset of the peaks from Figures 1A-1D or Figures 3A-3O, were generated.

[0054] The CART® software assigned a score to each biomarker in the subset, based on its relative importance. A score of 100 is very high and a score of 0 is very low. The CART® software also determined the sensitivity and specificity of each decision tree.

[0055] The data generated by the decision tree analysis was subjected to further analysis. The biomarkers were ranked based on their average scores, which were determined by adding up a biomarker's scores for each decision tree in which it appeared, and dividing by the total number of decision trees in which the biomarker appeared. Approximately 500 of the potential biomarkers showed up in at least one tree, and most of the biomarkers showed up in about 150 to 400 of the two thousand trees. The top 50 biomarkers for the IMAC and WCX chip arrays as determined by this method are shown in Figures 2 and 4A-4B, respectively.

[0056] All of the trees having sensitivities and specificities greater than 85% also were identified. All trees capable of distinguishing lung cancer from normal and having from 1 to 6 nodes that meet the 85/85 criterion are shown in Tables 1 and 2.

TABLE 1. Decision trees with IMAC Biomarkers.

2 Nodes				
474	151			
474	156			
522	507		2 trees	
522	440		2 trees	
3 Nodes				
266	454	474		
474	156	153		
474	40	156		
520	276	113		
520	265	401		
522	151	474		
522	478	153		
522	156	474		
4 Nodes				
148	521	508	251	

266	544	474	493	
266	157	126	420	
266	544	474	482	
266	471	474	38	
266	544	474	38	
266	514	471	203	
522	58	266	474	
5 Nodes				
266	544	473	151	437
266	454	474	153	264
273	143	544	401	199

TABLE 2. Decision Trees with WCX Biomarkers.

1 Node					
446					
447					
2 Nodes					
282	127				
3 Nodes					
61	16	27			
61	119	154			
61	120	154			
61	369	184			
61	184	129			
61	19	282			
133	282	319			
282	59	218			
282	111	65			

446	19	16			
4 Nodes					
61	369	282	184		
61	48	203	3		
446	369	111	67		
446	466	58	120		
446	19	59	113		
446	282	19	47		
447	118	59	417		
447	118	59	473		
447	65	59	275		
447	19	59	282		
447	369	59	206		
447	19	59	253		
447	19	47	70		
5 Nodes					
61	369	128	184	197	
61	17	425	366	341	
133	139	363	216	273	
282	133	48	19	253	
369	310	19	109	384	
446	282	15	319	66	
447	19	71	473	31	
447	19	17	473	438	
447	47	31	365	59	
6 Nodes					
369	366	192	471	19	439

[0057] Each of the biomarker combinations of Tables 1 and 2 are preferred combinations for distinguishing lung cancer subjects from normal subjects in accordance with the present invention.

[0058] All biomarkers that appeared in at least two of the trees that met the 85/85 criterion were identified. For these biomarkers, Tables 3 and 4 provide the number of times the biomarker occurred in a trees that met the criterion, as well as the ranking of that biomarker on the top 50 lists of Figures 2 and 4A-4B.

TABLE 3. Correlation of IMAC biomarker decision tree frequencies and ranking.

Peak		# times		Rank
266		9		9
522		8		1
474		4		14
520		2		3
148		1		8
273		1		2

TABLE 4. Correlation of WCX biomarker decision tree frequencies and ranking.

Peak		# times		Rank
447		11		2
61		10		1
446		7		3
282		4		9
369		2		8
133		2		4

[0059] Biomarkers that occurred frequently in the highly discriminatory trees occurred among the top 50 ranked biomarkers, and typically had a top 10 ranking. In addition, certain pairs of biomarkers reappear, *e.g.*, WM-447 and WM-59, WM-447 and WM-19, WM-19 and WM-59, IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-522 and IM-266. There also are repeats among triplets of biomarkers, such as IM-266, IM-266 and IM-38, and WM-447, WM-19 and WM-473. Other repeating pairs and trios of biomarkers can be seen in Tables 3 and 4, and are preferred.

[0060] Biomarkers and combinations of biomarkers identified in accordance with the present description may be used to qualify lung cancer status in a subject. In particular, a biomarker or combination of biomarkers can be used to distinguish lung cancer patients from normal patients with a high degree of specificity or sensitivity, *i.e.*, greater than at least 85%, preferably greater than at least 90%, and more preferably greater than 95%.

[0061] According to one aspect of the invention, therefore, the detection of biomarkers for diagnosis of lung cancer status entails contacting a sample from a subject with a substrate, e.g., a SELDI probe, having an adsorbent thereon, under conditions that allow binding between the biomarker and the adsorbent, and then detecting the biomarker bound to the adsorbent by gas phase ion spectrometry, for example, mass spectrometry. Other detection paradigms that can be employed to this end include optical methods, electrochemical methods (voltametry and amperometry techniques), atomic force microscopy, and radio frequency methods, *e.g.*, multipolar resonance spectroscopy. Illustrative of optical methods, in addition to microscopy, both confocal and non-confocal, are detection of fluorescence, luminescence, chemiluminescence, absorbance, reflectance, transmittance, and birefringence or refractive index (e.g., surface plasmon resonance, ellipsometry, a resonant mirror method, a grating coupler waveguide method or interferometry).

[0062] In one aspect, the markers of this invention are detect by gas phase ion spectrometry, which refers to the use of a gas phase ion spectrometer to detect gas phase ions. A gas phase ion spectrometer is an apparatus that detects gas phase ions. Gas phase ion spectrometers include an ion source that supplies gas phase ions. Gas

phase ion spectrometers include, for example, mass spectrometers, ion mobility spectrometers, and total ion current measuring devices.

[0063] "Mass spectrometer" refers to a gas phase ion spectrometer that measures a parameter which can be translated into mass-to-charge ratios of gas phase ions. Mass spectrometers generally include an ion source and a mass analyzer. Examples of mass spectrometers are time-of-flight, magnetic sector, quadrupole filter, ion trap, ion cyclotron resonance, electrostatic sector analyzer and hybrids of these. "Mass spectrometry" refers to the use of a mass spectrometer to detect gas phase ions. "Laser desorption mass spectrometer" refers to a mass spectrometer which uses laser as a means to desorb, volatilize, and ionize an analyte.

[0064] "Mass analyzer" refers to a sub-assembly of a mass spectrometer that comprises means for measuring a parameter which can be translated into mass-to-charge ratios of gas phase ions. In a time-of flight mass spectrometer the mass analyzer comprises an ion optic assembly, a flight tube and an ion detector.

[0065] "Ion source" refers to a sub-assembly of a gas phase ion spectrometer that provides gas phase ions. In one embodiment, the ion source provides ions through a desorption/ionization process. Such embodiments generally comprise a probe interface that positionally engages a probe in an interrogatable relationship to a source of ionizing energy (e.g., a laser desorption/ionization source) and in concurrent communication at atmospheric or subatmospheric pressure with a detector of a gas phase ion spectrometer.

[0066] Forms of ionizing energy for desorbing/ionizing an analyte from a solid phase include, for example: (1) laser energy; (2) fast atoms (used in fast atom bombardment); (3) high energy particles generated via beta decay of radionucleides (used in plasma desorption); and (4) primary ions generating secondary ions (used in secondary ion mass spectrometry). The preferred form of ionizing energy for solid phase analytes is a laser (used in laser desorption/ionization), in particular, nitrogen lasers, Nd-Yag lasers and other pulsed laser sources. "Fluence" refers to the laser energy delivered per unit area of interrogated image. Typically, a sample is placed on the surface of a probe, the probe is engaged with the probe interface and the probe

surface is struck with the ionizing energy. The energy desorbs analyte molecules from the surface into the gas phase and ionizes them.

[0067] Other forms of ionizing energy for analytes include, for example: (1) electrons which ionize gas phase neutrals; (2) strong electric field to induce ionization from gas phase, solid phase, or liquid phase neutrals; and (3) a source that applies a combination of ionization particles or electric fields with neutral chemicals to induce chemical ionization of solid phase, gas phase, and liquid phase neutrals.

[0068] A preferred mass spectrometric technique for use in the invention is Surface Enhanced Laser Desorption and Ionization (SELDI), as described, for example, in U.S. patents No. 5,719,060 and No. 6,225,047, both to Hutchens and Yip, in which the surface of a probe that presents the analyte (here, one or more of the biomarkers) to the energy source plays an active role in desorption/ionization of analyte molecules. In this context, "probe" refers to a device adapted to engage a probe interface and to present an analyte to ionizing energy for ionization and introduction into a gas phase ion spectrometer, such as a mass spectrometer. A probe typically includes a solid substrate, either flexible or rigid, that has a sample-presenting surface, on which an analyte is presented to the source of ionizing energy.

[0069] One version of SELDI, called Surface-Enhanced Affinity Capture" or "SEAC," involves the use of probes comprised of a chemically selective surface ("SELDI probe"). A "chemically selective surface" is one to which is bound either the adsorbent, also called a "binding moiety" or "capture reagent," or a reactive moiety that is capable of binding a capture reagent, *e.g.*, through a reaction forming a covalent or coordinate covalent bond.

[0070] The phrase "reactive moiety" here denotes a chemical moiety that is capable of binding a capture reagent. Epoxide and carbodiimidizole are useful reactive moieties to covalently bind polypeptide capture reagents such as antibodies or cellular receptors. Nitriloacetic acid and iminodiacetic acid are useful reactive moieties that function as chelating agents to bind metal ions that interact non-covalently with histidine containing peptides. A "reactive surface" is a surface to which a reactive moiety is bound. An "adsorbent" or "capture reagent" can be any material capable of

binding a biomarker of the invention. Suitable adsorbents for use in SELDI, according to the invention, are described in U.S. patent No. 6,225,047, *supra*.

[0071] One type of adsorbent is a "chromatographic adsorbent," which is a material typically used in chromatography. Chromatographic adsorbents include, for example, ion exchange materials, metal chelators, immobilized metal chelates, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, simple biomolecules (e.g., nucleotides, amino acids, simple sugars and fatty acids), mixed mode adsorbents (*e.g.*, hydrophobic attraction/electrostatic repulsion adsorbents). "Biospecific adsorbent" is another category, for adsorbents that contain a biomolecule, *e.g.*, a nucleotide, a nucleic acid molecule, an amino acid, a polypeptide, a polysaccharide, a lipid, a steroid or a conjugate of these (*e.g.*, a glycoprotein, a lipoprotein, a glycolipid). In certain instances the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Illustrative biospecific adsorbents are antibodies, receptor proteins, and nucleic acids. A biospecific adsorbent typically has higher specificity for a target analyte than a chromatographic adsorbent.

[0072] Another version of SELDI is Surface-Enhanced Neat Desorption (SEND), which involves the use of probes comprising energy absorbing molecules that are chemically bound to the probe surface ("SEND probe"). The phrase "Energy absorbing molecules" (EAM) denotes molecules that are capable of absorbing energy from a laser desorption ionization source and, thereafter, contributing to desorption and ionization of analyte molecules in contact therewith. The EAM category includes molecules used in MALDI , frequently referred to as "matrix;" and is exemplified by cinnamic acid derivatives, sinapinic acid (SPA), cyano-hydroxy-cinnamic acid (CHCA) and dihydroxybenzoic acid, ferulic acid, and hydroxyaceto-phenone derivatives. The category also includes EAMs used in SELDI, as enumerated, for example, by U.S. 5,719,060 and U.S. 60/351,971 (Kitagawa), filed January 25, 2002.

[0073] Another version of SELDI, called Surface-Enhanced Photolabile Attachment and Release (SEPAR), involves the use of probes having moieties attached to the surface that can covalently bind an analyte, and then release the analyte through breaking a photolabile bond in the moiety after exposure to light, *e.g.*, to laser light.

For instance, see U.S. 5,719,060. SEPAR and other forms of SELDI are readily adapted to detecting a biomarker or biomarker profile, pursuant to the present invention.

[0074] The detection of the biomarkers according to the invention can be enhanced by using certain selectivity conditions, *e.g.*, adsorbents or washing solutions. The phrase "wash solution" refers to an agent, typically a solution, which is used to affect or modify adsorption of an analyte to an adsorbent surface and/or to remove unbound materials from the surface. The elution characteristics of a wash solution can depend, for example, on pH, ionic strength, hydrophobicity, degree of chaotropism, detergent strength, and temperature.

[0075] Pursuant to one aspect of the present invention, a sample is analyzed by means of a "biochip," a term that denotes a solid substrate, having a generally planar surface, to which a capture reagent (adsorbent) is attached. Frequently, the surface of a biochip comprises a plurality of addressable locations, each of which has the capture reagent bound there. A biochip can be adapted to engage a probe interface and, hence, function as a probe in gas phase ion spectrometry preferably mass spectrometry. Alternatively, a biochip of the invention can be mounted onto another substrate to form a probe that can be inserted into the spectrometer.

[0076] A variety of biochips is available for the capture of biomarkers, in accordance with the present invention, from commercial sources such as Ciphergen Biosystems (Fremont, CA), Perkin Elmer (Packard BioScience Company (Meriden CT), Zyomyx (Hayward, CA), and Phylos (Lexington, MA). Exemplary of these biochips are those described in U.S. patents No. 6,225,047, supra, and No. 6,329,209 (Wagner et al.), and in PCT publications WO 99/51773 (Kuimelis and Wagner) and WO 00/56934 (Englert *et al.*).

[0077] More specifically, biochips produced by Ciphergen Biosystems have surfaces, presented on an aluminum substrate in strip form, to which are attached, at addressable locations, chromatographic or biospecific adsorbents. The surface of the strip is coated with silicon dioxide.

[0078] Illustrative of Ciphergen ProteinChip® arrays are biochips H4, SAX-2, WCX-2, and IMAC-3, which include a functionalized, cross-linked polymer in the

form of a hydrogel, physically attached to the surface of the biochip or covalently attached through a silane to the surface of the biochip. The H4 biochip has isopropyl functionalities for hydrophobic binding. The SAX-2 biochip has quaternary ammonium functionalities for anion exchange. The WCX-2 biochip has carboxylate functionalities for cation exchange. The IMAC-3 biochip has nitriloacetic acid functionalities that adsorb transition metal ions, such as Cu++ and Ni++, by chelation. These immobilized metal ions, in turn, allow for adsorption of biomarkers by coordinate covalent bonding. Thus, Ciphergen's IMAC ProteinChip® arrays are sold with reactive moieties that become adsorbent upon the addition by the user of a metal solution.

[0079] In keeping with the above-described principles, a substrate with an adsorbent is contacted with the sample, containing serum, for a period of time sufficient to allow biomarker that may be present to bind to the adsorbent. In one embodiment of the invention, more than one type of substrate with adsorbent thereon is contacted with the biological sample. For example, a sample may be applied to both a WCX and an IMAC chip. This technique can allow for even more definitive assessment of cancer status. After the incubation period, the substrate is washed to remove unbound material. Any suitable washing solutions can be used; preferably, aqueous solutions are employed.

[0080] An energy absorbing molecule then is applied to the substrate with the bound biomarkers. As noted, an energy absorbing molecule is a molecule that absorbs energy from an energy source such as a laser, thereby assisting in desorption of biomarkers from the substrate. Exemplary energy absorbing molecules include, as noted above, cinnamic acid derivatives, sinapinic acid and dihydroxybenzoic acid. Preferably sinapinic acid is used.

[0081] The biomarkers bound to the substrates are detected in a gas phase ion spectrometer such as a time-of-flight mass spectrometer. The biomarkers are ionized by an ionization source such as a laser, the generated ions are collected by an ion optic assembly, and then a mass analyzer disperses and analyzes the passing ions. The detector then translates information of the detected ions into mass-to-charge

ratios. Detection of a biomarker typically will involve detection of signal intensity. Thus, both the quantity and mass of the biomarker can be determined.

[0082] Data generated by desorption and detection of biomarkers can be analyzed with the use of a programmable digital computer. The computer program analyzes the data to indicate the number of markers detected, and optionally the strength of the signal and the determined molecular mass for each biomarker detected. Data analysis can include steps of determining signal strength of a biomarker and removing data deviating from a predetermined statistical distribution. For example, the observed peaks can be normalized, by calculating the height of each peak relative to some reference. The reference can be background noise generated by the instrument and chemicals such as the energy absorbing molecule which is set as zero in the scale.

[0083] The computer can transform the resulting data into various formats for display. The standard spectrum can be displayed, but in one useful format only the peak height and mass information are retained from the spectrum view, yielding a cleaner image and enabling biomarkers with nearly identical molecular weights to be more easily seen. In another useful format, two or more spectra are compared, conveniently highlighting unique biomarkers and biomarkers that are up- or down-regulated between samples. Using any of these formats, one can readily determine whether a particular biomarker is present in a sample.

[0084] Software used to analyze the data can include code that applies an algorithm to the analysis of the signal to determine whether the signal represents a peak in a signal that corresponds to a biomarker according to the present invention. The software also can subject the data regarding observed biomarker peaks to classification tree or ANN analysis, to determine whether a biomarker peak or combination of biomarker peaks is present that indicates lung cancer status. Analysis of the data may be "keyed" to a variety of parameters that are obtained either directly or indirectly from the mass spectrometric analysis of the sample. These parameters include, but are not limited to, the presence or absence of one or more peaks, the height of one or more peaks, the log of the height of one or more peaks, and other arithmetic manipulations of peak height data.

[0085] In another aspect, the present invention provides kits for aiding in the diagnosis of lung cancer status, which kits are used to detect biomarkers according to the invention. The kits screen for the presence of biomarkers and combinations of biomarkers that are differentially present in samples from normal subjects and subjects with lung cancer.

[0086] In one embodiment, the kit comprises a substrate having an adsorbent thereon, wherein the adsorbent is suitable for binding a biomarker according to the invention, and a washing solution or instructions for making a washing solution, in which the combination of the adsorbent and the washing solution allows detection of the biomarker using gas phase ion spectrometry, e.g., mass spectrometry. The kit may include more than type of adsorbent, each present on a different substrate.

[0087] In another embodiment, a kit of the invention may include a first substrate, comprising an adsorbent thereon, and a second substrate onto which the first substrate is positioned to form a probe, which can be inserted into a gas phase ion spectrometer, e.g., a mass spectrometer. In another embodiment, an inventive kit may comprise a single substrate that can be inserted into the spectrometer.

[0088] In a further embodiment, such a kit can comprise instructions for suitable operational parameters in the form of a label or separate insert. For example, the instructions may inform a consumer how to collect the sample or how to wash the probe. In yet another embodiment the kit can comprise one or more containers with biomarker samples, to be used as standard(s) for calibration.

[0089] In a preferred embodiment, the detection of biomarkers for diagnosis of lung cancer in a subject entails contacting a sample from a subject or patient, preferably a serum sample, with a substrate having an adsorbent thereon under conditions that allow binding between the biomarker and the adsorbent, and then detecting the biomarker bound to the adsorbent by gas phase ion spectrometry, preferably by Surface Enhanced Laser Desorption/Ionization (SELDI) mass spectrometry. The biomarkers are ionized by an ionization source such as a laser. The generated ions are collected by an ion optic assembly and accelerated toward an ion detector. Ions that strike the detector generate an electric potential that is digitized by a high speed time-array recording device that digitally captures the analog signal. Ciphergen's ProteinChip® system employs an analog-to-digital converter (ADC) to accomplish this. The ADC integrates detector output at regularly spaced time intervals into time-dependent bins. The time intervals typically are one to four nanoseconds long. Furthermore, the time-of-flight spectrum ultimately analyzed typically does not represent the signal from a single pulse of ionizing energy against a sample, but rather the sum of signals from a number of pulses. This reduces noise and increases dynamic range. This time-of-flight data is then subject to data processing. In Ciphergen's ProteinChip® software, data processing typically includes TOF-to-M/Z transformation, baseline subtraction, high frequency noise filtering. Thus, both the quantity and mass of the biomarker can be determined.

[0090] The detection of the biomarkers can be enhanced by using certain selectivity conditions, *e.g.*, adsorbents or washing solutions. In one embodiment, the same or similar selectivity conditions that were used to discover the biomarkers are used in the method of detecting the biomarker in the sample. For example, immobilized metal affinity capture chips such as the Cu(II) IMAC3 and weak cationic exchange chips such as the WCX2 chips are preferred as the adsorbents for biomarker detection. However, other adsorbents can be used, as long as they have the binding characteristics suitable for binding the biomarkers.

[0091] More particularly, armed with the information regarding the biomarkers identified herein, various methods can be used to recognize patterns of doublets, triplets, and higher combinations of biomarkers according to the invention. These methods take raw data regarding which peaks are present and their intensity and provide a differential diagnosis of lung cancer versus normal for a sample.

[0092] Thus, the process can be divided into the learning phase and the classification phase. In the learning phase, a learning algorithm is applied to a data set that includes members of the different classes that are meant to be classified, for example, data from a plurality of samples diagnosed as cancer and data from a plurality of samples assigned a negative diagnosis. The methods used to analyze the data include, but are not limited to, artificial neural network, support vector machines, genetic algorithm and self-organizing maps and classification and regression tree analysis. These methods are described, for example, in WO 01/31579, May 3, 2001

(Barnhill *et al.*); WO 02/06829, January 24, 2002 (Hitt *et al.*) and WO 02/42733, May 30, 2002 (Paulse *et al.*). The learning algorithm produces a classifying algorithm. The classifier is keyed to elements of the data, such as particular markers and particular intensities of markers, usually in combination, that can classify an unknown sample into one of the two classes. The classifier is ultimately used for diagnostic testing.

[0093] Software, both freeware and proprietary software, is readily available to analyze such patterns in data, and to devise additional patterns with any predetermined criteria for success. Those biomarkers which by themselves are predictive of a differential diagnosis of lung cancer versus normal do not require pattern recognition software to analyze the data.

[0094] The following examples are offered by way of illustration, and are not limiting.

Example I. Fractionation of serum

Buffers:

1. U9 (9M urea, 2% CHAPS, 50mM Tris-HCl pH9)
2. U1 (1M urea, 0.22% CHAPS, 50mM Tris-HCl pH9)
3. wash buffer 1: 50mM Tris-HCl with 0.1% n-octyl β-D-Glucopyranoside (OGP) pH9
4. wash buffer 2: 100mM sodium phosphate with 0.1% OGP pH7
5. wash buffer 3: 100mM sodium acetate with 0.1% OGP pH5
6. wash buffer 4: 100mM sodium acetate with 0.1% OGP pH4
7. wash buffer 5: 50mM sodium citrate with 0.1% OGP pH3
8. wash buffer 6: 33.3% isopropanol / 16.7% acetonitrile / 0.1% trifluoroacetic acid in water.

[0095] Thirty microliters of U9 buffer were added to 20μL of serum in a tube and were mixed at 4°C for 20 minutes. Ion exchange resin (Q Ceramic HyperDF ion exchange resin, Biosepra SA, France) was washed 3 times with 5 bed volumes of 50mM Tris-HCl pH9 and stored in 50% suspension. To each well of a 96-well filter plate (96-well Silent Screen filter plate, Loprodyne membrane, 0.45 micron pore,

Nalge Nunc International, USA), 125 μL of ion exchange resin (50% suspension) was added on a Biomek 2000 Automation Workstation (Beckman Coulter, Fullerton, CA), washed 3 times with 150μL U1 buffer, and vacuum dried. Urea-treated serum was transferred to each well of ion exchange resin. The serum tube was rinsed with 50μL of U1 buffer, which was also transferred to the corresponding well in filter plate. The filter plate was mixed on a platform shaker at 4°C for 30 minutes. Flow-through fraction was collected in a 96-well plate by vacuum suction (Fraction 1). Then, 100μL of wash buffer 1 was added to each well of filter plate and mixed for 10 minutes at room temperature. Eluant was collected into the same 96-well plate (Fraction 1). Resins in the filter plate were subsequently washed two times each with 100μL wash buffers 2, 3, 4, 5 and 6. Each eluant (total volume of 200μL) was collected in a 96-well plate (Fractions 2,3,4,5 and 6).

Example 2. SELDI analysis of fractionated serum

[0096] ProteinChip® Arrays were set up in 96-well bioprocessors. Buffer delivery and sample incubation were performed on a Biomek 2000 Automation Workstation. Each serum fraction was analyzed on IMAC3 (loaded with copper) and WCX2 ProteinChip® Arrays in duplicates. IMAC3 copper and WCX2 arrays (Ciphergen Biosystems Inc, Fremont, CA) were equilibrated two times with 150μL of binding buffer (100mM sodium phosphate + 0.5M NaCl pH7 for IMAC3, 100mM sodium acetate pH4 for WCX2). Each serum fraction was diluted in the corresponding binding buffer (1/5 dilution for IMAC3 and 1/10 dilution for WCX2) and 100μL was applied to each ProteinChip® array. Incubation was performed on a platform shaker at room temperature for 30 minutes. Each array was washed three times with 150μL of corresponding binding buffer and rinsed two times with water. ProteinChip® arrays were air-dried. Sinapinic acid matrix (prepared in 50% acetonitrile, 0.5% trifluoroacetic acid) was applied to each array. ProteinChip® arrays were read on a ProteinChip® PBSII Reader (Ciphergen Biosystems Inc.) A total of 253 laser shots were averaged for each array.

[0097] All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if

each individual publication or patent document were so individually denoted. By their citation of various references in this document Applicants do not admit that any particular reference is "prior art" to their invention.

What we claim is:

1. A method for qualifying lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a diagnostic level of a protein selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310,

wherein the biomarker is differentially present in samples of a subject with lung cancer and a normal subject that is free of lung cancer.

2. The method according to claim 1, wherein the protein is selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, and IM-155,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, and WM-70.

3. The method according to claim 1, wherein the protein is selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, and IM-454,

or from a second group consisting

(ii) WM-61, WM-447, WM-446, WM-133, and WM-119.

4. The method according to claim 1, which uses a single biomarker selected from the group consisting of the WM-446 and WM-447.

5. A method for qualifying lung carcinoma risk in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

6. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a pair of peaks selected either from a first group consisting of

(i) IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-266 and IM-522, IM- 266 and IM-544, IM-266 and IM-471, IM-474 and IM-151, IM-474 and IM-156, IM-474 and IM-544, IM-474 and IM-38, IM-522 and IM-507, IM-522 and IM-156, and IM-522 and IM-440;

or from a second group consisting of

(ii) WM-447 and WM-59, WM-447 and WM-19, WM-447 and WM-118, WM-447 and WM-473, WM-19 and WM-59, WM-19 and WM-473, WM-19 and WM-369, WM-61 and WM-154, WM-61 and WM-369, WM-118 and WM-59 and WM-282 and WM-127.

7. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a pair of peaks selected from either a first group consisting of

(i) IM-266 and IM-474, IM-266 and IM-544, and IM-156 and IM-522;

or from a second group consisting of

(ii) WM-447 and WM-59, WM-447 and WM-19, and WM-19 and WM-59.

8. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a triplet of peaks selected from

(i) IM-266, IM-454 and IM-474; and IM-266, IM-474 and IM-544;

or wherein the analysis is keyed to

(ii) WM-447, WM-19 and WM-473.

9. A kit for detecting and diagnosing lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

10. A kit according to claim 9, further comprising a washing solution or instructions for making a washing solution.

11. A kit according to claim 9, wherein the substrate is a SELDI probe that comprises either (i) functionalities that adsorb transition metal ions by chelation or (ii) functionalities that allow for cation exchange.

12. A method for qualifying lung adenocarcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429.

13. The method according to claim 12, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, and WM-120.

14. The method according to claim 12, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290.

15. A method for qualifying status of lung adenocarcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-

389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429.

16. The method according to claim 15, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, and WM-120.

17. The method according to claim 15, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290.

18. A kit for detecting and diagnosing lung adenocarcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of biomarkers selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

19. A kit according to claim 18, further comprising a washing solution or instructions for making a washing solution.

20. A kit according to claim 18, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

21. A method for qualifying squamous cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290,

WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128.

22. The method according to claim 21, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, and WM-70.

23. The method according to claim 21, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, and WM-133.

24. A method for qualifying status of squamous cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290, WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128.

25. The method according to claim 24, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, and WM-70.

26. The method according to claim 24, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, and WM-133.

27. A kit for detecting and diagnosing squamous cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290, WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

28. A kit according to claim 27, further comprising a washing solution or instructions for making a washing solution.

29. A kit according to claim 27, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

30. A method for qualifying small cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279.

31. The method according to claim 30, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, and WM-685.

32. The method according to claim 30, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, and WM-61.

33. A method for qualifying status of small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279.

34. The method according to claim 33, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, and WM-685.

35. The method according to claim 33, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, and WM-61.

36. A kit for detecting and diagnosing small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

37. A kit according to claim 36, further comprising a washing solution or instructions for making a washing solution.

38. A kit according to claim 36, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

39. A method for qualifying non-small cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38.

40. The method according to claim 39, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, and WM-456.

41. The method according to claim 39, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, and WM-340.

42. A method for qualifying status of non-small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38.

43. The method according to claim 42, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, and WM-456.

44. The method according to claim 42, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, and WM-340.

45. A kit for detecting and diagnosing non-small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

46. A kit according to claim 45, further comprising a washing solution or instructions for making a washing solution.

47. A kit according to claim 45, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

48. A method for qualifying large cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488.

49. The method according to claim 48, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, and WM-447.

50. The method according to claim 48, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, and WM-647.

51. A method for qualifying status of large cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488.

52. The method according to claim 51, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, and WM-447.

53. The method according to claim 51, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, and WM-647.

54. A kit for detecting and diagnosing large cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-

545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

55. A kit according to claim 50, further comprising a washing solution or instructions for making a washing solution.

56. A kit according to claim 50, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

57. A method for distinguishing lung adenocarcinoma from squamous lung carcinoma in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, WM-211, WM-362, WM-133, WM-414, WM-277, WM-141, WM-64, WM-135, WM-447, WM-383, WM-338, WM-63, WM-142, WM-446, WM-186, WM-111, WM-445, WM-455, WM-276, WM-444, WM-181, WM-35, WM-285, WM-456, WM-39, WM-82, WM-17, WM-203, WM-83, WM-412, WM-96, WM-74, WM-457, WM-431, WM-340, and WM-49.

58. The method according to claim 57, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, and WM-211.

59. The method according to claim 57, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, and WM-347.

60. A method for distinguishing lung adenocarcinoma from squamous lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group

consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, WM-211, WM-362, WM-133, WM-414, WM-277, WM-141, WM-64, WM-135, WM-447, WM-383, WM-338, WM-63, WM-142, WM-446, WM-186, WM-111, WM-445, WM-455, WM-276, WM-444, WM-181, WM-35, WM-285, WM-456, WM-39, WM-82, WM-17, WM-203, WM-83, WM-412, WM-96, WM-74, WM-457, WM-431, WM-340, and WM-49.

61. The method according to claim 60, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, and WM-211.

62. The method according to claim 60, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, and WM-347.

63. A kit for distinguishing lung adenocarcinoma from squamous lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

64. A kit according to claim 63, further comprising a washing solution or instructions for making a washing solution.

65. A kit according to claim 63, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

66. A method for distinguishing lung adenocarcinoma from small cell lung carcinoma in a subject, comprised of analyzing a biological sample from said subject

for a level of a protein selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, WM-455, WM-313, WM-456, WM-86, WM-70, WM-246, WM-360, WM-190, WM-418, WM-83, WM-257, WM-138, WM-47, WM-252, WM-282, WM-60, WM-68, WM-325, WM-402, WM-411, WM-405, WM-75, WM-417, WM-387, WM-26, WM-410, WM-420, WM-164, WM-67, WM-66, WM-391, WM-340, WM-428, WM-198, WM-312, and WM-152.

67. The method according to claim 66, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, and WM-455.

68. The method according to claim 66, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, and WM-79.

69. A method for distinguishing lung adenocarcinoma from small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, WM-455, WM-313, WM-456, WM-86, WM-70, WM-246, WM-360, WM-190, WM-418, WM-83, WM-257, WM-138, WM-47, WM-252, WM-282, WM-60, WM-68, WM-325, WM-402, WM-411, WM-405, WM-75, WM-417, WM-387, WM-26, WM-410, WM-420, WM-164, WM-67, WM-66, WM-391, WM-340, WM-428, WM-198, WM-312, and WM-152.

70. The method according to claim 69, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, and WM-455.

71. The method according to claim 69, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79.

72. A kit for distinguishing lung adenocarcinoma from small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

73. A kit according to claim 72, further comprising a washing solution or instructions for making a washing solution.

74. A kit according to claim 72, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

75. A method for distinguishing squamous cell lung carcinoma from small cell lung carcinoma in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37.

76. The method according to claim 75, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, and WM-447.

77. The method according to claim 75, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, and WM-363.

78. A method for distinguishing squamous cell lung carcinoma from small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37.

79. The method according to claim 78, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, and WM-447.

80. The method according to claim 78, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, and WM-363.

81. A kit for distinguishing squamous cell lung carcinoma from small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

82. A kit according to claim 81, further comprising a washing solution or instructions for making a washing solution.

83. A kit according to claim 81, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

84. Software for qualifying lung carcinoma status in a subject, comprising an algorithm for analyzing data extracted from a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, wherein said data relates to one or more biomarkers selected from either a first group consisting of:

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

85. Software according to claim 84, wherein said algorithm carries out a pattern-recognition analysis that is keyed to data relating to at least one of the biomarkers.

86. Software according to claim 85, wherein said algorithm comprises classification tree analysis that is keyed to data relating to at least one of the biomarkers.

87. Software according to claim 85, wherein said algorithm comprises artificial neural network analysis that is keyed to data relating to at least one of the biomarkers.

88. A method for qualifying lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a diagnostic level of a biomarker that is serum amyloid A protein or a fragment thereof.

89. A method according to claim 88, wherein said serum biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons.

90. A method according to claim 89, wherein said serum biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons.

91. A method according to claim 88, wherein said serum biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

92. A method according to claim 88, for qualifying risk of lung adenocarcinoma.

93. A method according to claim 88, for qualifying risk of squamous cell lung carcinoma.

94. A method according to claim 88, for qualifying risk of small cell lung carcinoma.

95. A method according to claim 88, for qualifying risk of non-small cell lung carcinoma.

96. A method according to claim 88, for qualifying risk of large cell lung carcinoma.

97. A kit for detecting and diagnosing lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers that are serum amyloid A protein or a fragment thereof.

and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

98. A kit according to claim 97, wherein said serum biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons.

99. A kit according to claim 98, wherein said serum biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons.

100. A kit according to claim 97, wherein said serum biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

101. A kit according to claim 97, further comprising a washing solution or instructions for making a washing solution.

102. A kit according to claim 97, wherein the substrate is a SELDI probe.

FIGURE 1A

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-1	2011	A	IM-37	3893	A	IM-72	54026	A	IM-109	2882	B
IM-2	2030	A	IM-38	3960	A	IM-73	60170	A	IM-110	2967	B
IM-3	2069	A	IM-39	3972	A	IM-75	74372	A	IM-111	2977	B
IM-4	2128	A	IM-40	3984	A	IM-76	75545	A	IM-112	2994	B
IM-5	2146	A	IM-41	4066	A	IM-77	77543	A	IM-113	3031	B
IM-6	2186	A	IM-42	4178	A	IM-78	79507	A	IM-114	3048	B
IM-7	2232	A	IM-43	4287	A	IM-79	89854	A	IM-115	3148	B
IM-8	2277	A	IM-44	4297	A	IM-80	101831	A	IM-116	3166	B
IM-9	2295	A	IM-45	4309	A	IM-81	104301	A	IM-117	3283	B
IM-10	2318	A	IM-46	4484	A	IM-82	125160	A	IM-118	3308	B
IM-11	2411	A	IM-47	4649	A	IM-83	132976	A	IM-119	3332	B
IM-12	2434	A	IM-48	4798	A	IM-84	149099	A	IM-120	3432	B
IM-13	2467	A	IM-49	5104	A	IM-85	2016	B	IM-121	3450	B
IM-14	2482	A	IM-50	5918	A	IM-86	2029	B	IM-122	3561	B
IM-15	2498	A	IM-51	6122	A	IM-87	2144	B	IM-123	3615	B
IM-16	2565	A	IM-52	6192	A	IM-88	2130	B	IM-124	3714	B
IM-17	2574	A	IM-53	6452	A	IM-89	2168	B	IM-125	3730	B
IM-18	2586	A	IM-54	6660	A	IM-90	2184	B	IM-126	3834	B
IM-19	2605	A	IM-55	7768	A	IM-91	2200	B	IM-127	3899	B
IM-20	2722	A	IM-56	8145	A	IM-92	2284	B	IM-128	3969	B
IM-21	2746	A	IM-57	8954	A	IM-93	2289	B	IM-129	3986	B
IM-22	2788	A	IM-58	9312	A	IM-94	2314	B	IM-130	3997	B
IM-23	2855	A	IM-59	9449	A	IM-95	2414	B	IM-131	4013	B
IM-24	2871	A	IM-60	10272	A	IM-96	2428	B	IM-132	4181	B
IM-25	2984	A	IM-61	11683	A	IM-97	2451	B	IM-133	4297	B
IM-26	3030	A	IM-62	13376	A	IM-98	2468	B	IM-134	4311	B
IM-27	3144	A	IM-63	14698	A	IM-99	2483	B	IM-135	4465	B
IM-28	3243	A	IM-64	15190	A	IM-100	2565	B	IM-136	4484	B
IM-29	3273	A	IM-64	66758	A	IM-101	2583	B	IM-137	4579	B
IM-30	3290	A	IM-65	15951	A	IM-102	2597	B	IM-138	4608	B
IM-31	3369	A	IM-66	15172	A	IM-103	2697	B	IM-139	4669	B
IM-32	3445	A	IM-67	15925	A	IM-104	2715	B	IM-140	4747	B
IM-33	3483	A	IM-68	23436	A	IM-105	2740	B	IM-141	4862	B
IM-34	3676	A	IM-69	39794	A	IM-106	2752	B	IM-142	4891	B
IM-35	3779	A	IM-70	44166	A	IM-107	2767	B	IM-143	5033	B
IM-36	3793	A	IM-71	46890	A	IM-108	2865	B	IM-144	5077	B

FIGURE 1B

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-145	5099	B	IM-181	16018	B	IM-217	2130	C	IM-253	3733	C
IM-146	5143	B	IM-182	17346	B	IM-218	2145	C	IM-254	3833	C
IM-147	5158	B	IM-183	18311	B	IM-219	2167	C	IM-255	3900	C
IM-148	5272	B	IM-184	22586	B	IM-220	2182	C	IM-256	4010	C
IM-149	5306	B	IM-185	23422	B	IM-221	2199	C	IM-257	4145	C
IM-150	5349	B	IM-186	27969	B	IM-222	2211	C	IM-258	4187	C
IM-151	5364	B	IM-187	33283	B	IM-223	2230	C	IM-259	4299	C
IM-152	5421	B	IM-188	39808	B	IM-224	2250	C	IM-260	4466	C
IM-153	5554	B	IM-189	43110	B	IM-225	2280	C	IM-261	4582	C
IM-154	5711	B	IM-190	44454	B	IM-226	2297	C	IM-262	4813	0
IM-155	5876	B	IM-191	47215	B	IM-227	2317	C	IM-263	4876	C
IM-156	5916	B	IM-192	53784	B	IM-228	2412	C	IM-264	5032	C
IM-157	5931	B	IM-193	55952	B	IM-229	2428	C	IM-265	5347	C
IM-158	5988	B	IM-194	60573	B	IM-230	2468	C	IM-266	5365	C
IM-159	6137	B	IM-195	66346	B	IM-231	2481	C	IM-267	5932	C
IM-160	6200	B	IM-196	73387	B	IM-232	2498	C	IM-268	7767	C
IM-161	6443	B	IM-197	79203	B	IM-233	2567	C	IM-269	7973	C
IM-162	6644	B	IM-198	89302	B	IM-234	2585	C	IM-270	8143	C
IM-163	6958	B	IM-199	94226	B	IM-235	2599	C	IM-271	9187	C
IM-164	7481	B	IM-200	99358	B	IM-236	2698	C	IM-272	9293	C
IM-165	7568	B	IM-201	102096	B	IM-237	2715	C	IM-273	11705	C
IM-166	7765	B	IM-202	107199	B	IM-238	2745	C	IM-274	14041	C
IM-167	7955	B	IM-203	116936	B	IM-239	2786	C	IM-275	15114	C
IM-168	8144	B	IM-204	119487	B	IM-240	2867	C	IM-276	15939	C
IM-169	8698	B	IM-205	122103	B	IM-241	2885	C	IM-277	22321	C
IM-170	8821	B	IM-206	125431	B	IM-242	2998	C	IM-278	28001	C
IM-171	8944	B	IM-207	132052	B	IM-243	3052	C	IM-279	33296	C
IM-172	9138	B	IM-208	138518	B	IM-244	3096	C	IM-280	39770	C
IM-173	9298	B	IM-209	145147	B	IM-245	3151	C	IM-281	44460	C
IM-174	9390	B	IM-210	157602	B	IM-246	3167	C	IM-282	47307	C
IM-175	9516	B	IM-211	168579	B	IM-247	3286	C	IM-283	50625	C
IM-176	11711	B	IM-212	173391	B	IM-248	3303	C	IM-284	55898	C
IM-177	11914	B	IM-213	2011	C	IM-249	3335	C	IM-285	60882	C
IM-178	14033	B	IM-214	2030	C	IM-250	3448	C	IM-286	66294	C
IM-179	15110	B	IM-215	2050	C	IM-251	3619	C	IM-287	78892	C
IM-180	15838	B	IM-216	2096	C	IM-252	3709	C	IM-288	83848	C

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-289	89081	C	IM-325	2565	D	IM-361	13857	D	IM-397	2082	E
IM-290	94147	C	IM-326	2582	D	IM-362	14056	D	IM-398	2128	E
IM-291	99324	C	IM-327	2597	D	IM-363	15108	D	IM-399	2148	E
IM-292	107163	C	IM-328	2716	D	IM-364	15844	D	IM-400	2170	E
IM-293	110350	C	IM-329	2747	D	IM-365	22243	D	IM-401	2187	E
IM-294	113339	C	IM-330	2767	D	IM-366	25465	D	IM-402	2206	E
IM-295	116291	C	IM-331	2866	D	IM-367	28022	D	IM-403	2232	E
IM-296	122769	C	IM-332	2882	D	IM-368	33272	D	IM-404	2250	E
IM-297	131908	C	IM-333	2894	D	IM-369	40149	D	IM-405	2279	E
IM-298	145248	C	IM-334	3032	D	IM-370	43113	D	IM-406	2296	E
IM-299	159252	C	IM-335	3050	D	IM-371	44219	D	IM-407	2314	E
IM-300	165164	C	IM-336	3148	D	IM-372	47196	D	IM-408	2354	E
IM-301	174928	C	IM-337	3164	D	IM-373	51062	D	IM-409	2394	E
IM-302	196003	C	IM-338	3278	D	IM-374	56082	D	IM-410	2413	E
IM-303	2007	D	IM-339	3334	D	IM-375	58239	D	IM-411	2436	E
IM-304	2016	D	IM-340	3385	D	IM-376	60285	D	IM-412	2457	E
IM-305	2030	D	IM-341	3432	D	IM-377	66148	D	IM-413	2466	E
IM-306	2052	D	IM-342	3451	D	IM-378	73668	D	IM-414	2489	E
IM-307	2099	D	IM-343	3617	D	IM-379	77433	D	IM-415	2566	E
IM-308	2130	D	IM-344	3701	D	IM-380	79986	D	IM-416	2583	E
IM-309	2144	D	IM-345	3725	D	IM-381	80844	D	IM-417	2612	E
IM-310	2154	D	IM-346	3833	D	IM-382	88962	D	IM-418	2662	E
IM-311	2166	D	IM-347	3899	D	IM-383	94399	D	IM-419	2723	E
IM-312	2184	D	IM-348	4008	D	IM-384	99419	D	IM-420	2738	E
IM-313	2204	D	IM-349	4157	D	IM-385	108395	D	IM-421	2750	E
IM-314	2231	D	IM-350	4297	D	IM-386	116433	D	IM-422	2849	E
IM-315	2252	D	IM-351	4580	D	IM-387	123337	D	IM-423	2867	E
IM-316	2275	D	IM-352	4805	D	IM-388	131977	D	IM-424	3036	E
IM-317	2299	D	IM-353	6946	D	IM-389	145658	D	IM-425	3147	E
IM-318	2316	D	IM-354	7053	D	IM-390	152603	D	IM-426	3281	E
IM-319	2412	D	IM-355	7767	D	IM-391	159524	D	IM-427	3319	E
IM-320	2435	D	IM-356	7954	D	IM-392	196072	D	IM-428	3445	E
IM-321	2466	D	IM-357	8139	D	IM-393	2010	E	IM-429	3693	E
IM-322	2480	D	IM-358	9292	D	IM-394	2029	E	IM-430	3731	E
IM-323	2499	D	IM-359	11671	D	IM-395	2050	E	IM-431	3818	E
IM-324	2518	D	IM-360	13727	D	IM-396	2068	E	IM-432	3885	E

FIGURE 1D

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-433	4136	E	IM-469	86211	E	IM-505	4174	F	IM-541	95033	F
IM-434	4169	E	IM-470	89407	E	IM-506	4362	F	IM-542	100310	F
IM-435	4257	E	IM-471	100270	E	IM-507	4473	F	IM-543	116889	F
IM-436	4277	E	IM-472	109638	E	IM-508	4631	F	IM-544	132711	F
IM-437	4355	E	IM-473	117132	E	IM-509	4822	F	IM-545	147276	F
IM-438	4369	E	IM-474	132843	E	IM-510	5862	F	IM-546	160768	F
IM-439	4470	E	IM-475	147160	E	IM-511	6192	F			
IM-440	4488	E	IM-476	152199	E	IM-512	6941	F			
IM-441	4541	E	IM-477	166461	E	IM-513	7626	F			
IM-442	4634	E	IM-478	176635	E	IM-514	7772	F			
IM-443	4841	E	IM-479	2011	F	IM-515	7957	F			
IM-444	5862	E	IM-480	2030	F	IM-516	8150	F			
IM-445	5911	E	IM-481	2128	F	IM-517	8954	F			
IM-446	6849	E	IM-482	2149	F	IM-518	9300	F			
IM-447	6952	E	IM-483	2186	F	IM-519	11545	F			
IM-448	7769	E	IM-484	2207	F	IM-520	11717	F			
IM-449	8148	E	IM-485	2279	F	IM-521	13887	F			
IM-450	8260	E	IM-486	2299	F	IM-522	14073	F			
IM-451	8785	E	IM-487	2319	F	IM-523	15196	F			
IM-452	9301	E	IM-488	2412	F	IM-524	15903	F			
IM-453	10071	E	IM-489	2434	F	IM-525	22460	F			
IM-454	11721	E	IM-490	2467	F	IM-526	23135	F			
IM-455	13910	E	IM-491	2485	F	IM-527	28135	F			
IM-456	15919	E	IM-492	2582	F	IM-528	33577	F			
IM-457	22422	E	IM-493	2605	F	IM-529	39813	F			
IM-458	28233	E	IM-494	2697	F	IM-530	42344	F			
IM-459	33490	E	IM-495	2751	F	IM-531	43274	F			
IM-460	43121	E	IM-496	2885	F	IM-532	44345	F			
IM-461	44558	E	IM-497	3036	F	IM-533	51007	F			
IM-462	46694	E	IM-498	3151	F	IM-534	56318	F			
IM-463	50954	E	IM-499	3372	F	IM-535	60079	F			
IM-464	54478	E	IM-500	3440	F	IM-536	66890	F			
IM-465	60041	E	IM-501	3488	F	IM-537	75122	F			
IM-466	66852	E	IM-502	3717	F	IM-538	78429	F			
IM-467	75580	E	IM-503	3890	F	IM-539	81249	F			
IM-468	79463	E	IM-504	4155	F	IM-540	89384	F			

FIGURE 2

RANK	MW	MARKER ID
1	14073	IM-522
2	11705	IM-273
3	11717	IM-520
4	11545	IM-519
5	11721	IM-454
6	4473	IM-507
7	13887	IM-521
8	5272	IM-148
9	5385	IM-266
10	75122	IM-537
11	100270	IM-471
12	5862	IM-510
13	132711	IM-544
14	132843	IM-474
15	5876	IM-155
16	5932	IM-157
17	11711	IM-176
18	5911	IM-445
19	11914	IM-177
20	4486	IM-440
21	79463	IM-468
22	4369	IM-438
23	100310	IM-542
24	11671	IM-359
25	4277	IM-436
26	2752	IM-106
27	13910	IM-455
28	5862	IM-444
29	5988	IM-158
30	5347	IM-265
31	5918	IM-50
32	6137	IM-159
33	5916	IM-156
34	4470	IM-439
35	5931	IM-157
36	4631	IM-508
37	7772	IM-514
38	176635	IM-478
39	117132	IM-473
40	13727	IM-360
41	4257	IM-435
42	5349	IM-150
43	5364	IM-151
44	2967	IM-110
45	6122	IM-51
46	6958	IM-163
47	4355	IM-437
48	160768	IM-546
49	5554	IM-153
50	7767	IM-268

Figure 3A

Peak ID	Fraction		Normal vs Cancer		Adeno vs Normal	Squamous vs Normal	Small Cell vs Normal	Non-small Cell vs Normal	Large Cell vs Normal		Adeno vs Squamous	Adeno vs Small Cell	Squamous vs Small Cell
WM-1	A		2010		2011	2010	2011	2009	2011		2009	2010	2010
WM-2	A		2032		2030	2031		2032	2032		2031		
WM-3	A		2052						2052				
WM-4	A		2088		2089	2089	2087	2082	2088		2089	2089	2089
WM-5	A		2098		2097	2089	2096	2078	2098		2090	2096	2096
WM-6	A		2168			2169		2170	2165				2169
WM-7	A		2233						2233				
WM-8	A		2277		2275	2274	2278	2277	2276		2277	2277	2277
WM-9	A		2298		2296	2294	2298	2298	2298		2298	2298	2298
WM-10	A		2398					2347	2398				
WM-11	A		2498		2500	2500	2499	2501	2483		2501	2499	2500
WM-12	A		2582		2580	2580	2584	2581	2582		2581	2582	2581
WM-13	A		2723						2723			2723	
WM-14	A		2738		2740	2740	2741	2740	2738		2738	2740	2739
WM-15	A		2751		2752	2752	2753	2753	2752		2752	2752	2752
WM-16	A		2768		2767	2769	2768		2768		2768	2768	2769
WM-17	A		2788		2788	2789	2790	2788	2788		2788	2785	2785
WM-18	A		2803		2804	2804	2800	2843	2804		2806	2804	2805
WM-19	A		2832		2822	2832	2833	2831	2832		2812	2832	2831
WM-20	A		2854		2852	2853	2858		2852		2852	2854	2853
WM-21	A		3082		3082	3078	3083	3081	3084		3084	3084	
WM-22	A		3147		3155	3157	3160	3160	3160		3155	3158	3158
WM-23	A		3187		3180	3190	3186	3189	3188		3180	3191	3189
WM-24	A		3215		3217	3218	3219	3218	3216		3216	3213	3214
WM-25	A		3240			3240			3244		3235	3236	3239
WM-26	A		3294		3292	3292	3294	3298	3298		3293	3293	3294
WM-27	A		3277		3274	3273	3276				3277	3274	3277
WM-28	A		3290		3288	3287	3288	3289	3290		3290	3291	3290
WM-29	A		3319		3318	3317	3329	3314	3318		3317	3317	3347
WM-30	A		3369		3365	3369	3372	3368	3369		3369	3371	3371
WM-31	A		3440		3437	3438	3441	3442	3438		3440	3440	3440
WM-32	A		3524		3529	3529	3528	3534	3527		3524	3524	3524
WM-33	A		3683		3683	3682	3684	3683	3683		3682	3683	3683
WM-34	A		3774		3776	3778	3776		3774			3770	3776
WM-35	A		3815		3816	3815	3816	3817	3818		3814	3816	3817
WM-36	A		3892		3892	3890	3893	3891	3891		3892	3892	3892
WM-37	A		3957		3959	3957	3960	3957	3956		3956	3956	3957
WM-38	A		3971		3976	3972	3976	3973	3973		3972	3974	3972
WM-39	A		4087		4089	4084	4088	4047	4086		4088	4089	
WM-40	A		4174		4175	4173	4178		4177			4178	4176
WM-41	A		4283		4285	4283	4286	4283	4283		4283	4283	4283
WM-42	A		4303		4305	4301	4301	4305	4301		4303	4303	4304
WM-43	A		4478		4478	4478	4476	4478	4478		4478	4478	4478
WM-44	A		4664		4661	4654	4660	4651	4660		4653	4651	4664
WM-45	A		5020		5024	5020	5016	5023	5020				
WM-46	A		5445		5448	5442	5439	5443	5444				
WM-47	A		5911		5903	5911	5905	5911	5910		5913	5911	5913
WM-48	A		6122		6125	6125	6126	6127	6126		6118	6118	6118
WM-49	A		6420		6442	6444	6443	6442	6442		6443	6441	6444
WM-50	A		6637		6638	6639	6640	6639	6639		6640	6637	6639

Figure 3B

WM-51	A	6827	6830	6829	6831	6829	6829			
WM-52	A	7581	7584	7591	7588	7546	7548	7574		7879
WM-53	A	7788	7783	7785	7784	7784	7768	7769	7770	7770
WM-54	A	8141	8139	8135	8133	8137	8138	8141	8142	8143
WM-55	A	8611	8611	8609	8604	8608	8610	8614	8616	8615
WM-56	A	8844	8842	8846	8843	8845	8846	8846	8847	8848
WM-57	A	9142	9159	9143	9143	9148	9144	9142	9164	9152
WM-58	A	9303	9302	9300	9305	9301	9302	9298	9301	9301
WM-59	A	10078	10080	10076	10080	10077	10080		10078	
WM-60	A	10278	10270	10277	10278	10279	10270	10271	10238	10280
WM-61	A	10851	10867	10869	10852	10861	10844	10859	10862	10859
WM-62	A	11494	11490	11490	11490			11490	11490	11462
WM-63	A	11960	11960	11963	11764			11956	11957	11951
WM-64	A	12865	12877	12879	12862	12861	12878	12879	12880	12868
WM-65	A	13336		13324	13335			13339	13341	13336
WM-66	A	14698	14701	14700	14702	14700	14695	14731	14730	14763
WM-67	A	15142	15147	15148	15139	15163	15134	15144	15145	15142
WM-68	A	15891	15885	15880	15878	15881	15896	15892	15882	15880
WM-69	A	23472	23443	23462	23438	23449	23463	23465	23473	23469
WM-70	A	28117	28163	28080	28104	28083	28092	28120	28146	
WM-71	A	28787	28781	28739	28764	28753	28746			
WM-72	A	33374	33339	33360	33341	33373	33303	33339	33380	33408
WM-73	A	38853	38871	38842	38851	38839	38911	38848	38894	38838
WM-74	A	44438	44327	44484	44383	44338	44280	44538	44430	44482
WM-75	A	50187	49914	50049	49849			50125	49980	50037
WM-76	A	51328	51314	51317	51279	51304	51285	51349	51444	51175
WM-77	A	53780	53780	53866	53803	53710	53675	53824	53868	54027
WM-78	A	56030							56033	
WM-79	A	60096	60039	60010	60019	60013	60080	60103	60103	60306
WM-80	A	62238	62237	62108	62571	62239	62238		62510	62237
WM-81	A	63010	62929	62357		62980	62996	63049		63102
WM-82	A	66003	66635	67068	66608	66686	66660	66640	65870	66571
WM-83	A	68400	68432	68577	68358		68581	67923	68363	68533
WM-84	A	74708	74529	74862	74941	74315	74497	74663	74811	74025
WM-85	A	79106	79062	79081	79089	79029	79091	79076	79149	79224
WM-86	A	80339	89830	89518	80072	80198	80027	80365	80220	80354
WM-87	A	101107	100885	101117	100963	100984	100636	101180	101081	101106
WM-88	A	113053	112840	113182	112874	112891	112142	113000	113012	
WM-89	A	124363	124229	124261	124232	124847	124225	124317	124330	124629
WM-90	A	134813	134770	134483	134478	134111	134836	134748	134811	134691
WM-91	A	148915	147888	147834	147803	147893	147803	148122	147988	148127
WM-92	A	172246	171388	171728	171418	171879	171101	172342	172147	171869
WM-93	B	2011	2011	2013	2012	2012	2013	2010	2011	2012
WM-94	B	2097	2087					2089	2087	2089
WM-95	B	2081	2079	2079	2080	2079	2079	2082	2081	2080
WM-96	B	2229	2229	2229	2227	2229	2227	2229	2229	2229
WM-97	B	2278	2278	2278	2277	2279	2278	2278	2277	2278
WM-98	B	2294	2293	2294	2298	2297	2296	2294	2298	2286
WM-99	B	2430	2429	2429	2429	2428	2429	2429	2429	2429
WM-100	B	2500	2501	2500	2509	2501	2500	2499	2496	2497
WM-101	B	2580	2580	2580	2580	2580	2580	2581	2581	2581

Figure 3C

WM-102	B	2723	2722	2722	2722	2722	2722	2723	2733	2733
WM-103	B	2763	2753	2763	2748	2763	2763	2752	2746	2747
WM-104	B	2865	2864	2886	2886	2885	2884	2886	2885	2886
WM-105	B	2933	2933	2933	2934	2934	2934	2933	2932	2933
WM-106	B	3062	3062	3062	3062	3063	3062	3063	3062	3063
WM-107	B	3158	3157	3158	3158	3157	3158	3158	3135	3165
WM-108	B	3286	3287	3285	3287	3287	3287	3288	3288	3289
WM-109	B	3311	3310	3311	3310	3310	3310	3317		
WM-110	B	3446	3447	3447	3446	3446	3446		3445	3446
WM-111	B	3617	3618	3618	3616	3619	3618	3618	3618	3616
WM-112	B	3880	3880	3880	3883	3884	3883	3880	3880	3880
WM-113	B	4210	4219	4216	4210	4211	4219	4212	4210	4211
WM-114	B	4292	4291	4283	4292	4292	4291		4291	4290
WM-115	B	4416	4419	4417	4414	4414	4414			
WM-116	B	4601	4600	4600				4597	4597	4596
WM-117	B	4868	4868	4864	4868	4863	4866	4864	4865	4866
WM-118	B	5013	5006	5010	5012	5012	5012			
WM-119	B	5342	5340	5342	5342	5344	5345	5340	5344	5342
WM-120	B	5808	5815	5810	5811	5814	5814	5811	5810	5810
WM-121	B	6440	6446	6440	6436	6439	6442	6436	6435	6434
WM-122	B	6636	6643	6638	6635	6637	6637	6636	6629	6637
WM-123	B	6822	6849	6843	6822	6842	6844	6845	6829	6830
WM-124	B	7673		7670	7587			7670		7673
WM-125	B	7768	7772	7769	7769	7763	7758	7768	7769	7770
WM-126	B	7942	7940	7930	7940			7943	7939	7941
WM-127	B	8142	8140	8142	8138	8139	8138	8143	8148	8143
WM-128	B	8700	8703	8693	8701	8701	8700	8699	8699	8696
WM-129	B	8822	8830	8823	8820	8824	8822	8822	8824	8823
WM-130	B	8846	8882	8945	8940	8944	8941	8845	8844	8845
WM-131	B	9186	9391	9186	9187	9184	9185	9196	9194	9188
WM-132	B	9296	9303	9295	9297	9297	9298	9293	9205	9294
WM-133	B	11820	11815	11513		11517		11615	11523	11632
WM-134	B	11721	11709	11701	11710	11737	11719	11708	11709	11701
WM-135	B	14062	14080	14083	14084	14082	14080	14082	14083	14083
WM-136	B	15132	15143	15139	15135	15133	15133	15136	15143	15141
WM-137	B	15882	15884	15873	15881	15879	15878	15872	15876	15879
WM-138	B	17344	17323	17296	17333	17331	17320	17302	17325	17307
WM-139	B	22287	22274	22288	22294	22291	22282	22275	22287	22373
WM-140	B	23360	23413	23446	23357	23380	23380	23440	23367	23420
WM-141	B	28114	28114	28117	28112	28110	28115	28117	28113	28114
WM-142	B	33349	33304	33358	33354	33357	33304	33369	33378	33394
WM-143	B	37634	37809	37831	37849	37889	37886	37588	37809	37853
WM-144	B	38880	38780	38857	38808	38718	38819	38880	38874	38808
WM-145	B	44580	44632	44591	44571	44586	44585	44627	44637	44580
WM-146	B	47400	47432	47419	47408	47407	47401	47433	47361	47422
WM-147	B	51287	51329	51287	51306	51259	51308	51323	51310	51309
WM-148	B	58233	58278	58289	58334	58287	58294	58251	58293	58277
WM-149	B	59869	59894	59842	59872	59808	59839	59896	59834	59848
WM-150	B	60480	60484	60471	60480	60451	60484	60480	60489	60459
WM-151	B	73122	73083	73086	73171	73080	73072	73080	73140	73180
WM-152	B	75411	75404	75482	75383	75400	75470	75284	75289	75317

Figure 3D

WAI-153	B	78775	78789	78791	78804	78804	78816	78783	78772	78803
WAI-154	B	88333	88326	88436	88215	88188	88201	88531	88256	88596
WAI-155	B	94857	94638	94634	94479	94481	94839	94834	94580	94533
WAI-156	B	99822	99871	100030	99968	99962	99848	100098	100005	100062
WAI-157	B	107011	107088	107058	107088	107031	107088	107052	107008	107089
WAI-158	B	110637	110703	110659	110398	110649	110693	110687	110323	110681
WAI-159	B	117723	117778	117880	117925		117804	117862	118033	117949
WAI-160	B	132592	132580	132587	132601	132849	132598	132802	132808	132612
WAI-161	B	144790	144818	144276	144884	144410	144428	144778	144783	145033
WAI-162	B	158637	158398	158694	158780	158438	159132	158958	159030	158871
WAI-163	B	178311	174626	174728	174603	174600	174974	173478	178620	176363
WAI-164	B	187100	187046	187670	187436	187031	187223	187086	187100	187052
WAI-165	C	2011	2010	2011	2011	2010	2010	2010	2011	2010
WAI-166	C	2031	2032	2029	2032	2032	2032	2032	2032	2032
WAI-167	C	2076	2078	2078	2077	2080	2077	2078	2078	2079
WAI-168	C	2188	2163	2188	2188	2188	2187	2187	2188	
WAI-169	C	2184	2183	2183	2183	2184	2184	2184	2183	2186
WAI-170	C	2211	2211	2210	2212	2211	2211	2211	2211	2216
WAI-171	C	2228	2229	2226	2226	2229	2228	2228	2229	2228
WAI-172	C	2276	2276	2281	2276	2276	2277	2276	2277	2272
WAI-173	C	2297	2298	2294	2302	2298	2298	2297	2297	2297
WAI-174	C	2352	2354	2354	2355	2354	2353	2353	2353	2353
WAI-175	C	2425	2429	2429	2431	2429	2427	2429	2427	2429
WAI-176	C	2474	2479	2480	2487	2480	2479	2474	2480	2474
WAI-177	C	2528	2539	2530	2542	2538	2536	2536	2536	2536
WAI-178	C	2582	2580	2581	2585	2583	2581	2580	2580	2582
WAI-179	C	2636	2636	2637	2636	2637	2636	2636	2636	2637
WAI-180	C	2746	2760	2769	2767	2789	2758	2751	2780	2761
WAI-181	C	2883	2884	2888	2884	2884	2883	2885	2884	2886
WAI-182	C	2934	2934	2935	2933	2936	2934	2935	2934	2933
WAI-183	C	3082	3083	3084	3080	3083	3081	3084	3082	3083
WAI-184	C	3144			3144					
WAI-185	C	3184	3151	3184	3185	3151	3151	3184	3183	3133
WAI-186	C	3283	3283	3283	3280	3283	3284	3282	3281	3282
WAI-187	C	3445	3446	3447	3480	3446	3443	3446	3441	3445
WAI-188	C	3565	3535	3567	3563	3638	3630	3564	3568	3554
WAI-189	C	3606	3685	3687	3688	3687	3685	3690	3665	3688
WAI-190	C	3813	3817	3817	3813	3814	3814	3811	3814	3813
WAI-191	C	3840	3830	3843	3844	3838	3836	3836	3842	3837
WAI-192	C	3946	3931	3952	3954	3953				
WAI-193	C	4141	4144	4132		4144	4134	4134	4141	4130
WAI-194	C	4208	4213	4214	4294	4213	4208	4210	4210	4211
WAI-195	C	4286	4289	4287		4291	4289	4286	4285	4287
WAI-196	C	4406	4417	4413		4418	4410	4410	4419	4410
WAI-197	C	4780	4784	4786		4793	4785	4789	4779	4801
WAI-198	C	6837	6832	6832	6816	6830	6837	6827	6834	6832
WAI-199	C	7771	7771	7774	7761	7767	7769	7774	7769	7775
WAI-200	C	8141	8151	8151	8174	8149	8149	8151	8139	8152
WAI-201	C	9196	9197	9196	9227	9197	9194	9204	9195	9196
WAI-202	C	9300	9308	9303		9305	9300	9298	9299	9299
WAI-203	C	11709		11704	11697	11695	11712	11705	11701	11705

Figure 3E

WAI-204	C	14083	14088	14090	14079	14059	14089	14088	14089	14089
WAI-205	C	15149	15141	15146	15139	15133	15136	15139	15139	15139
WAI-206	C	15863	15870	15880	15882	15867	15864	15867	15865	16866
WAI-207	C	17365	17387	17387	17376	17368	17368	17337		
WAI-208	C	22236	22263	22263	22250	22286	22232	22240	22251	22280
WAI-209	C	29116	29117	29123	29129	29129	29140	29110	29114	29129
WAI-210	C	33310	33315	33323	33326	33329	33318	33314	33312	33330
WAI-211	C	37250	37214	37276	37218	37278	37233	37236	37205	37270
WAI-212	C	44511	44626	44613	44515	44444	44603	44642	44539	44518
WAI-213	C	47380	47376	47434	47386	47403	47391	47384	47377	47423
WAI-214	C	51210	51236	51289	51264	51287	51297	51237	51289	51289
WAI-215	C	56284	56280	56240	56233	56176	56212	56269	56307	56336
WAI-216	C	58761	58763	58764	58736	58806	58710	58764	58752	58760
WAI-217	C	66456	66448	66448	66443	66391	66437	66463	66447	66462
WAI-218	C	74748	74814	74895	74703	74791	74769	74880	74623	74687
WAI-219	C	78618	78619	78890	78735	78835	78882	78809	78601	78846
WAI-220	C	80332	80406	80465	80375	80380	80276	80337	80772	80459
WAI-221	C	83866	83837	83809	83817	83887	83474	83604	83612	83436
WAI-222	C	88017	88029	88891	88373	88300	88083	88914	88930	88980
WAI-223	C	94673	94719	94702	94836	94834	94617	94725	94711	94736
WAI-224	C	99811	99696	99713	99738	99985	99881	99788	99783	99796
WAI-225	C	110774	110788	110804	110785	110825	110818	110629	110736	110818
WAI-226	C	116513	116483	116239	116397	116343	117805	116217	116066	116344
WAI-227	C	133223	133397	133440	133417	133634	133428	133417	132589	133435
WAI-228	C	145134	146097	145761	145842	145400	145167	145477	145817	145894
WAI-229	C	154812	154830	154787	154631	156094	154990	155076	155043	156076
WAI-230	C	166467	166373	166442	165369	165420	166362	166431	166374	166437
WAI-231	C	177303	177164	177360	177204	177365	177338	177059	177024	176877
WAI-232	C	181213								
WAI-233	D	196820	196068	197080	197000	196969	197090	196811	196871	197016
WAI-234	D	2011	2010	2011	2010	2009	2009	2010	2010	2012
WAI-235	D	2031	2032	2032	2032	2029	2030	2032	2031	2033
WAI-236	D	2054	2054	2052	2053	2050	2053	2050	2050	2054
WAI-237	D	2079	2079	2078	2080	2078	2077	2078	2078	2079
WAI-238	D	2159	2167	2167	2167	2167	2167	2169	2169	2167
WAI-239	D	2186		2186	2184	2185	2183		2185	2186
WAI-240	D	2211	2212	2211	2212	2209	2210	2211	2211	2212
WAI-241	D	2230	2233	2229	2231	2229	2229	2229	2229	2229
WAI-242	D	2269	2269	2255	2259	2254	2254	2250	2255	2256
WAI-243	D	2276	2277	2278	2277	2278	2273	2276	2278	2274
WAI-244	D	2302	2299	2298	2300	2297	2298	2299	2299	2299
WAI-245	D	2357	2354	2368	2354	2352	2354	2352	2353	2354
WAI-246	D	2427	2428	2428	2428	2427	2427	2427	2428	2428
WAI-247	D	2483	2481	2480	2483	2473	2479	2480	2481	2481
WAI-248	D	2500	2499	2498	2500	2498	2500	2500	2500	2498
WAI-249	D	2544	2539	2543	2531	2542	2543	2542		
WAI-250	D	2579				2579	2584			
WAI-251	D	2636	2637	2637	2637	2636	2637	2637	2637	2637
WAI-252	D	2756	2751	2749	2754	2749	2748	2748	2750	2750
WAI-253	D	2886	2885	2883	2887	2882	2883	2884	2884	2884
WAI-254	D	2936	2937	2985	2936	2936	2936	2936	2936	2936

Figure 3F

Figure 3G

Figure 3H

WM-357	E	8628	8629	8634	8629	8627	8628	8631	8630	8630
WM-358	E	8955	8948		8963	8952	8953	8945	8957	8946
WM-359	E	9170	9174	9181	9170	9170	9179	9177		9184
WM-360	E	9300	9302	9297	9299	9301	9299	9301	9297	9298
WM-361	E	9470	9470	9476	9484	9482	9480	9470	9469	9477
WM-362	E	11530	11532	11515		11520		11536	11527	11527
WM-363	E	11714	11705	11699	11697	11697	11690	11714	11703	11718
WM-364	E	12462	12462	12480	12464	12463	12467	12451	12460	12458
WM-365	E	12922	12914	12904	12913	12922	12913	12917	12928	12916
WM-366	E	13607	13667	13679	13668	13698	13671	13601	13683	13667
WM-367	E	15131	15140	15155	15144	15154	15150	15185	15166	15184
WM-368	E	15891	15891	15891	15891	15889	15894	15859	15896	15896
WM-369	E	17364	17365	17370	17360	17361	17362	17363	17361	17366
WM-370	E	22300	22309	22345	22349	22322	22314	22304	22309	22332
WM-371	E	28160	28183	28181	28182	28183	28181	28183	28154	28172
WM-372	E	33430	33437	33481	33431	33429	33391	33425	33413	33428
WM-373	E	38433		38235						38228
WM-374	E	44637	44603	44590	44627	44591	44578	44628	44641	44675
WM-375	E	61578	61389	61379	61357	53429	61308	61507	61480	61408
WM-376	E	65790	60221	60942	60839	59647	60744	60620	60747	58708
WM-377	E	66087	64563	66538	66572	66468	66427	66591	66580	65804
WM-378	E	75385	75333	75469	75338	75201	75188	75408	75436	75413
WM-379	E	78384	78373	78334	78336	78436	70767	78380	78378	78327
WM-380	E	89411	89514	89256	89480	89370	89337	89421	89542	89361
WM-381	E	100091	100264	99991	99679	99902	100034	100149	100029	100091
WM-382	E	110187	110138	109854	109894	109810	109772	110474	110414	109821
WM-383	E	117646	117783	117802	117746	117720	117918	117686	117802	117448
WM-384	E	132840	132829	132821	132806	132827	132570	132887	132843	132842
WM-385	E	148533	148371	148507	148563	148206	148470	148912	148019	148853
WM-386	E	154171	152836					152949	153495	152534
WM-387	E	165716	165965	165729	165182	165727	165884	165882	165929	165575
WM-388	E	182079	182281	182824	182289	182280	182217	183080	182701	183356
WM-389	E	187329	187192	187349	187208	187254	187285	187249	187213	187301
WM-390	F	2011	2013	2010	2010	2012	2011	2011	2011	2011
WM-391	F	2031	2033	2029	2028	2030	2029	2029	2029	2029
WM-392	F	2040	2056		2049	2053	2054		2042	
WM-393	F	2060	2069	2066	2047	2071	2072	2070	2071	2067
WM-394	F	2085	2083	2080	2083	2084	2084	2084	2084	2080
WM-395	F	2129	2129	2129	2128	2125		2130	2125	2128
WM-396	F	2148	2164	2167	2147	2167	2165	2157	2168	2166
WM-397	F	2214	2218	2219	2214	2214	2212	2213	2213	2213
WM-398	F	2234	2232	2234	2233	2234	2233	2234	2233	2234
WM-399	F	2280	2287	2279	2277	2280	2276	2279	2276	2279
WM-400	F	2296	2298	2298	2297	2297	2297	2297	2298	2299
WM-401	F	2360	2396		2380	2389			2398	
WM-402	F	2413	2411	2413	2413	2412	2413	2413	2413	2412
WM-403	F	2482	2462	2443	2479	2474	2482	2482	2481	2482
WM-404	F	2501	2500	2500	2500	2501	2500	2501	2531	2500
WM-405	F	2560	2569	2569	2570	2569	2569	2569	2569	2569
WM-406	F	2582	2581	2592	2582	2591	2582	2581	2582	2581
WM-407	F	2687	2687	2687	2700	2695	2698		2698	

Figure 3I

WM-408	F	2723	2722	2723	2723	2723	2724	2723	2722	2722
WM-409	F	2753	2751	2751	2751	2750	2761	2761	2752	2760
WM-410	F	2866	2866	2867	2860	2865	2866	2865	2868	2868
WM-411	F	2980	2979	2981	2984	2979	2980	2979	2979	2979
WM-412	F	3151	3156	3158	3160	3156	3137	3132	3153	3152
WM-413	F	3373	3371	3372	3369	3372	3370	3371	3371	3372
WM-414	F	3441	3441	3441	3441	3443	3444	3439	3439	3439
WM-415	F	3497	3494	3497	3486	3487	3487	3486	3480	3482
WM-416	F	3569	3570		–		3579	3579	3580	3569
WM-417	F	3729	3714	3712	3721	3717	3718	3713	3717	3717
WM-418	F	3881	3886				3867		3886	
WM-419	F	4007							4001	
WM-420	F	4055	4067	4054	4054			4054	4055	4044
WM-421	F	4165	4164	4156	4180	4186	4180	4154	4165	4165
WM-422	F	4174	4179		4180					
WM-423	F	4201	4199	4203	4209	4203	4202	4204	4202	4212
WM-424	F	4216	4219					4216	4216	
WM-425	F	4358	4359						4301	
WM-426	F	4379	4375	4374	4367	4369	4370		4376	4378
WM-427	F	4414	4421	4414	4420	4413	4414	4420	4419	4417
WM-428	F	4473	4472	4478	4474	4475	4472	4473	4471	4473
WM-429	F	4636	4631	4630	4635	4636	4635	4630	4630	4628
WM-430	F	4632	4632	4631	4625	4636	4632	4637	4634	4631
WM-431	F	5880		5861	5880			5864	5863	5863
WM-432	F	6447	6446	6463	6450	6466	6464	6448	6451	6449
WM-433	F	6455	6491		6500			6494	6493	6490
WM-434	F	6662	6660	6640	6654	6659	6657	6660	6659	6646
WM-435	F	6840	6854	6847	6848	6840	6850	6846	6840	6847
WM-436	F	7191	7202	7181	7194	7182	7186	7185	7183	7182
WM-437	F	7681	7600		7609					
WM-438	F	7774	7774	7771	7777	7770	7771	7772	7771	7771
WM-439	F	7946		7948	7950			7951	7951	7949
WM-440	F	8238	8244	8239	8236	8244	8242	8241	8244	8231
WM-441	F	8710	8718	8708	8712	8711	8712	8706	8706	8791
WM-442	F	8836	8834	8831	8835	8837	8834	8831	8830	8831
WM-443	F	8960	8962	8905	8958	8961	8959	8958	8859	8855
WM-444	F	9175	9174	9171	9181	9176	9174	9171	9171	9171
WM-445	F	9466	9476	9468	9491	9463	9463	9479	9477	9467
WM-446	F	11563	11553	11566	11573	11562	11564	11565	11564	11565
WM-447	F	11743	11728	11737	11729	11717	11710	11730	11729	11738
WM-448	F	12473	12480	12468	12487	12468	12431	12451	12465	12464
WM-449	F	12619	12629	12625	12627	12625	12626		12622	12625
WM-450	F	13909	13919	13903	13907	13907	13903	13911	13909	13909
WM-451	F	14097	14098	14101	14096	14049	14095	14101	14098	14099
WM-452	F	15162	15172	15161	15162	15148	15180	15181	15139	15180
WM-453	F	15340		15341	15335			15344	15340	15337
WM-454	F	15890	15883	15880	15888	15988	15984		15914	15903
WM-455	F	17302	17313	17306	17323	17319	17324	17298	17299	17350
WM-456	F	17365	17390	17394	17402	17408	17404	17391	17380	
WM-457	F	23401	23275	23344	23332	23334	23283	23353	23365	23398
WM-458	F	28290	28215	28193	28200	28219	28178	28185	28190	28194

Figure 3J

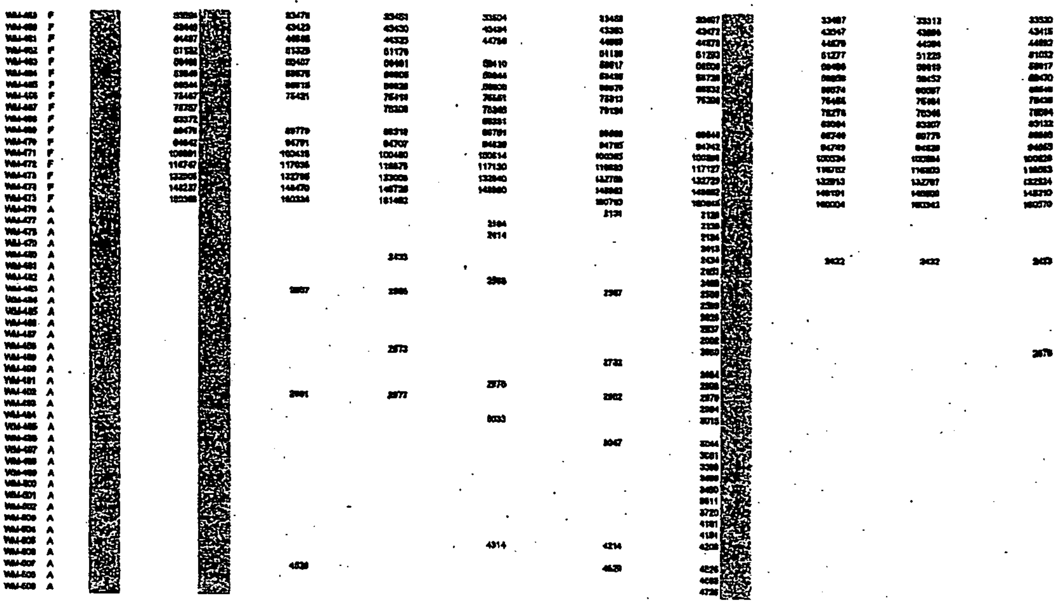


Figure 3K

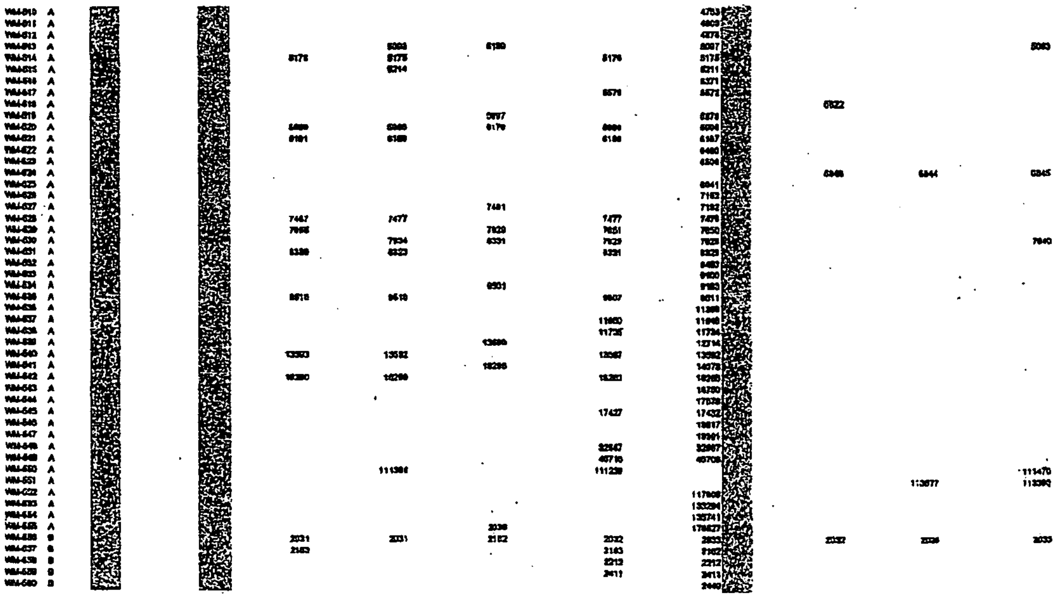


Figure 3L

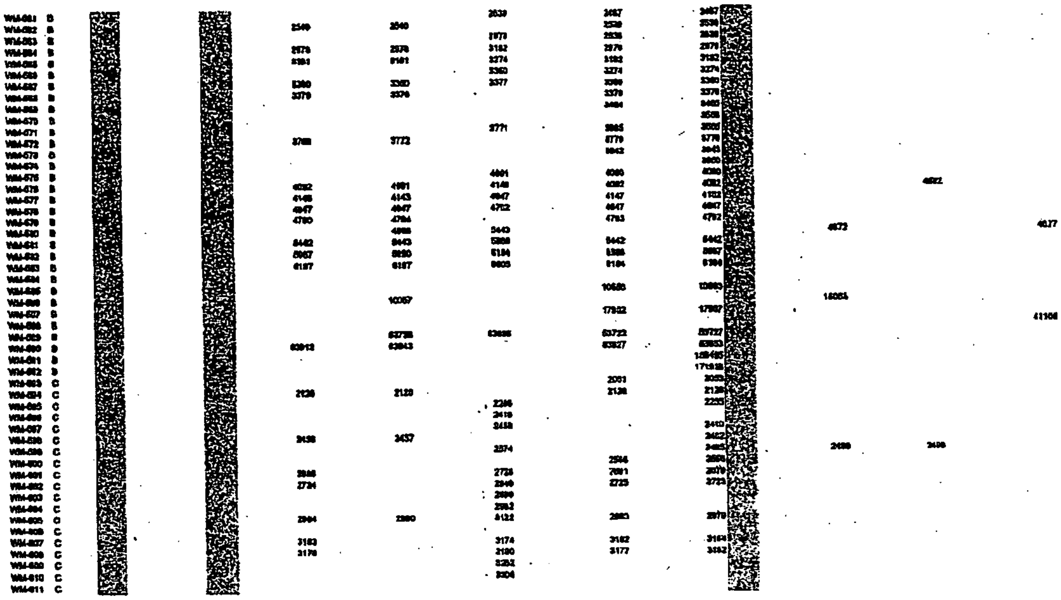


Figure 3M

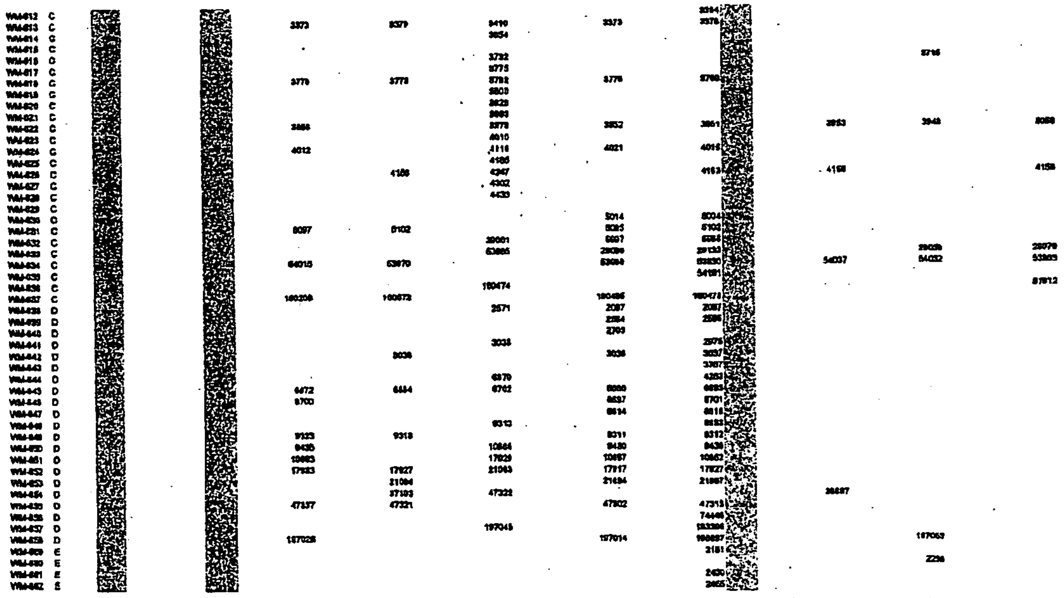


Figure 3N

WM-663	E					2590	2466	2467				
WM-664	E			2890	2890		2890	2890				
WM-665	E			2730	3722		2729	2722		2729	2723	2733
WM-666	E			2761	2761		2761	2731		2761	2761	2761
WM-667	E					3376		3085				
WM-668	E				3376	4131		3376				3370
WM-669	E			4129	4129	4162	4130			4129		4125
WM-670	E			4162	4165		4162	4181				4165
WM-671	E				4267							
WM-672	E				4345							
WM-673	E				4466						4430	
WM-674	E				4635							
WM-675	E						4694	4608				
WM-676	E									4544	4549	4537
WM-677	E					6310	5966	5964				
WM-678	E					6445	6310	6310				
WM-679	E			6446		6434	6442	6444				6343
WM-680	E			6461			6462	6461				6432
WM-681	E											7844
WM-682	E							10943				
WM-683	E						13604					
WM-684	E					17615		14406				
WM-685	E			17623	17620	40286	17669	17606				
WM-686	E						40601	40280				
WM-687	E						78293					
WM-688	E				61967	64150						62082
WM-689	E			64031		64883	64156	64159				
WM-690	E			64747	64749		64734	64880				
WM-691	E											144070
WM-692	E									164782		164983
WM-693	E						178344	178742				
WM-694	F						2384	2183				
WM-695	F			2281		3322						
WM-696	F			2326	3319	4172	3314	3318				
WM-697	F			4167	4176		4174	4172		4170	4169	4166
WM-698	F					5016	4719	4716				
WM-699	F			5011	5090		5011	5004				
WM-700	F				6679					6679		6679
WM-701	F				7579					7591	7579	7580
WM-702	F				7634		7623	7621				
WM-703	F							8311				
WM-704	F						14425	14421				
WM-705	F					17043				16962		
WM-706	F			17835	17943		17946	17941				
WM-707	F											46158
WM-708	F			45419	45389	50968	46391	46385				
WM-709	F			59619		58644				30514	59434	
WM-710	F							84602				
WM-711	F											60483
WM-712	F									79934		
WM-713	F			62174	62044							

Figure 3O.

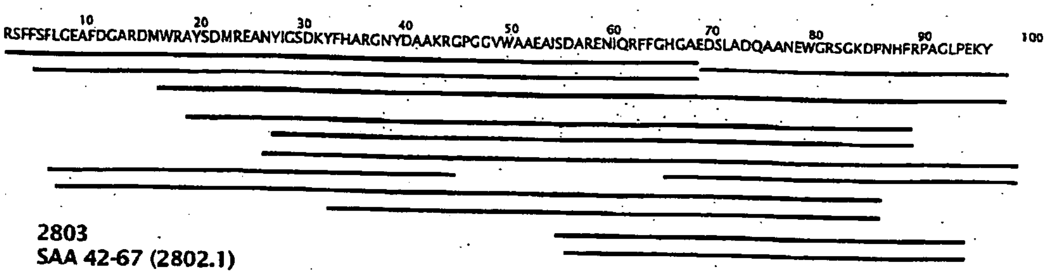
WM-714	F			64139	64472		64125	64106	
WM-715	F					161441	64712	64790	
WM-716	F						162354	163375	
WM-717	F							158686	

Figure 4A

Rank	Normal vs Cancer	Adeno vs Normal	Squamous vs Normal	Small Cell vs Normal	Non-small Cell vs Normal	Large Cell vs Normal	Adeno vs Squamous	Adeno vs Small Cell	Squamous vs Small Cell
1	WM-61	WM-447	WM-447	WM-70	WM-341	WM-16	WM-62	WM-457	WM-278
2	WM-447	WM-652	WM-61	WM-706	WM-342	WM-26	WM-415	WM-72	WM-277
3	WM-446	WM-61	WM-277	WM-389	WM-343	WM-409	WM-182	WM-389	WM-362
4	WM-133	WM-446	WM-446	WM-447	WM-48	WM-134	WM-365	WM-78	WM-257
5	WM-119	WM-290	WM-133	WM-61	WM-340	WM-647	WM-347	WM-79	WM-363
6	WM-278	WM-353	WM-134	WM-652	WM-348	WM-377	WM-134	WM-73	WM-347
7	WM-134	WM-133	WM-363	WM-282	WM-47	WM-310	WM-36	WM-64	WM-83
8	WM-363	WM-341	WM-362	WM-448	WM-339	WM-363	WM-108	WM-329	WM-254
9	WM-282	WM-285	WM-276	WM-456	WM-389	WM-448	WM-68	WM-419	WM-17
10	WM-342	WM-358	WM-706	WM-134	WM-659	WM-221	WM-151	WM-65	WM-252
11	WM-120	WM-282	WM-203	WM-203	WM-447	WM-648	WM-289	WM-62	WM-431
12	WM-290	WM-362	WM-466	WM-548	WM-652	WM-657	WM-383	WM-53	WM-613
13	WM-65	WM-610	WM-369	WM-458	WM-184	WM-290	WM-61	WM-412	WM-446
14	WM-277	WM-292	WM-65	WM-65	WM-587	WM-328	WM-117	WM-449	WM-355
15	WM-70	WM-120	WM-70	WM-686	WM-456	WM-447	WM-211	WM-453	WM-447
16	WM-369	WM-134	WM-341	WM-473	WM-450	WM-634	WM-302	WM-313	WM-133
17	WM-17	WM-278	WM-429	WM-343	WM-283	WM-183	WM-139	WM-458	WM-245
18	WM-473	WM-428	WM-347	WM-468	WM-207	WM-190	WM-414	WM-66	WM-52
19	WM-47	WM-277	WM-17	WM-361	WM-436	WM-696	WM-277	WM-79	WM-96
20	WM-203	WM-20	WM-47	WM-340	WM-384	WM-307	WM-141	WM-248	WM-236
21	WM-278	WM-119	WM-431	WM-383	WM-61	WM-466	WM-64	WM-380	WM-243
22	WM-279	WM-340	WM-62	WM-339	WM-167	WM-20	WM-135	WM-180	WM-138
23	WM-62	WM-48	WM-473	WM-457	WM-302	WM-17	WM-447	WM-418	WM-62
24	WM-358	WM-359	WM-334	WM-66	WM-285	WM-545	WM-363	WM-83	WM-580
25	WM-458	WM-450	WM-436	WM-508	WM-650	WM-47	WM-336	WM-257	WM-134
26	WM-425	WM-47	WM-652	WM-72	WM-203	WM-191	WM-63	WM-138	WM-240
27	WM-384	WM-343	WM-282	WM-287	WM-119	WM-147	WM-142	WM-47	WM-258
28	WM-287	WM-17	WM-369	WM-62	WM-282	WM-489	WM-446	WM-252	WM-203
29	WM-420	WM-583	WM-290	WM-528	WM-686	WM-590	WM-166	WM-282	WM-111
30	WM-292	WM-70	WM-279	WM-65	WM-383	WM-218	WM-111	WM-60	WM-96
31	WM-431	WM-706	WM-456	WM-73	WM-429	WM-285	WM-445	WM-68	WM-247
32	WM-453	WM-348	WM-673	WM-436	WM-11	WM-652	WM-455	WM-329	WM-157
33	WM-20	WM-465	WM-340	WM-384	WM-208	WM-651	WM-276	WM-402	WM-242
34	WM-348	WM-648	WM-65	WM-63	WM-451	WM-386	WM-444	WM-411	WM-658
35	WM-19	WM-384	WM-455	WM-450	WM-473	WM-403	WM-181	WM-406	WM-63
36	WM-389	WM-338	WM-645	WM-310	WM-220	WM-418	WM-35	WM-75	WM-239
37	WM-63	WM-294	WM-138	WM-277	WM-685	WM-430	WM-286	WM-417	WM-234
38	WM-436	WM-339	WM-420	WM-79	WM-338	WM-466	WM-458	WM-867	WM-274

Figure 4B

39	WM-450	WM-473	WM-450	WM-207	WM-71	WM-714	WM-39	WM-25	WM-370
40	WM-468	WM-389	WM-369	WM-278	WM-288	WM-646	WM-62	WM-410	WM-301
41	WM-298	WM-39	WM-279	WM-290	WM-70	WM-189	WM-17	WM-420	WM-449
42	WM-343	WM-283	WM-342	WM-385	WM-545	WM-302	WM-203	WM-184	WM-74
43	WM-341	WM-685	WM-471	WM-472	WM-675	WM-567	WM-63	WM-67	WM-261
44	WM-339	WM-86	WM-674	WM-420	WM-448	WM-578	WM-412	WM-68	WM-467
45	WM-88	WM-55	WM-120	WM-147	WM-120	WM-131	WM-86	WM-391	WM-237
46	WM-65	WM-650	WM-20	WM-66	WM-287	WM-706	WM-74	WM-340	WM-262
47	WM-48	WM-307	WM-287	WM-689	WM-468	WM-338	WM-457	WM-428	WM-295
48	WM-36	WM-278	WM-83	WM-357	WM-347	WM-309	WM-431	WM-186	WM-288
49	WM-138	WM-342	WM-154	WM-429	WM-153	WM-65	WM-340	WM-312	WM-384
50	WM-310	WM-429	WM-128	WM-279	WM-38	WM-486	WM-49	WM-152	WM-37



2803
SAA 42-67 (2802.1)

3168
SAA 69-97 (3167.3)

3277
SAA 39-68 (3276.6)

3552
SAA 38-70 (3552)

3897
SAA 64-98 (3897.2)

4300
SAA 54-93 (4302.5)

4490
SAA 53-93 (4489)

4655
SAA 5-44 (4655.0)

5927
SAA 32-85 (5925.3)

6874
SAA 26-88 (6873.3)

7776
SAA 1-68 (7774.6)

7941
SAA 18-88 (7939.5)

8152
SAA 25-98 (8150)

8952
SAA 6-85 (8950)

9233
SAA 16-*97* (9235)

10300
SAA 6-*97* (10299.1)

10866
SAA 4-101 (10871.8)

10851
SAA 5-102 (10853.7)

Figure 5

Figure 6

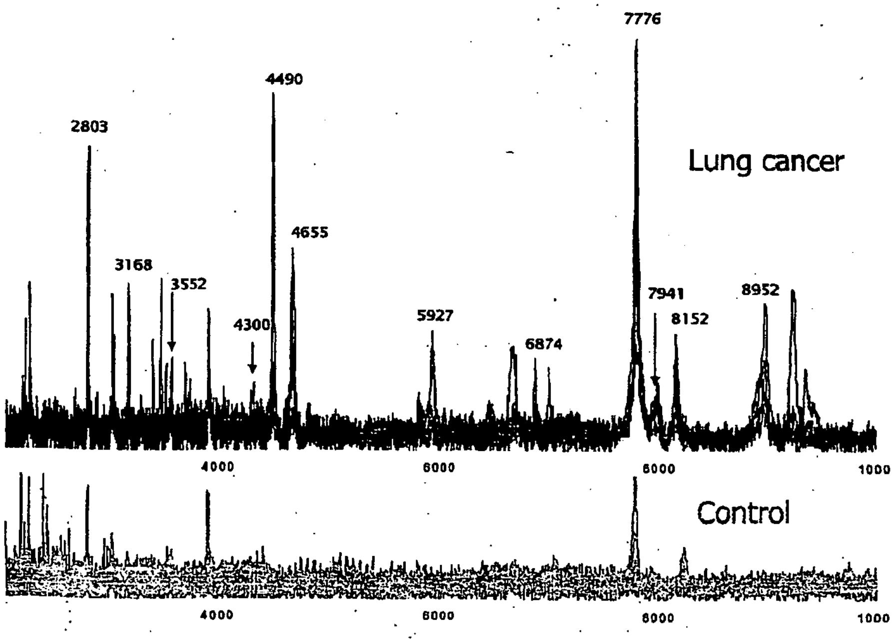


FIGURE 7
Protein Profile of Selected Samples Q Fraction 1 WCX2

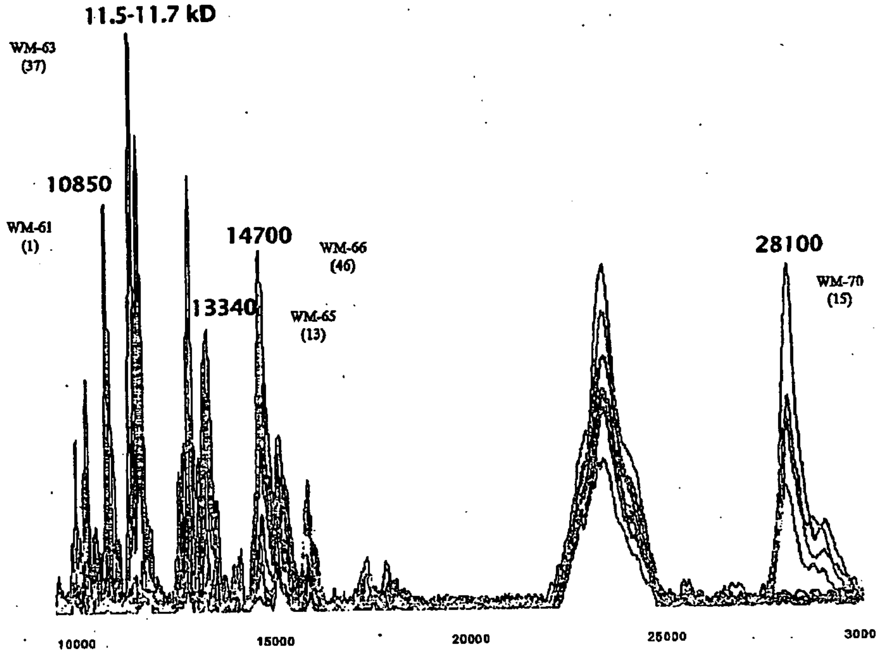


Figure 8

Protein Profile of Selected Samples Q Fraction 1 WCX2

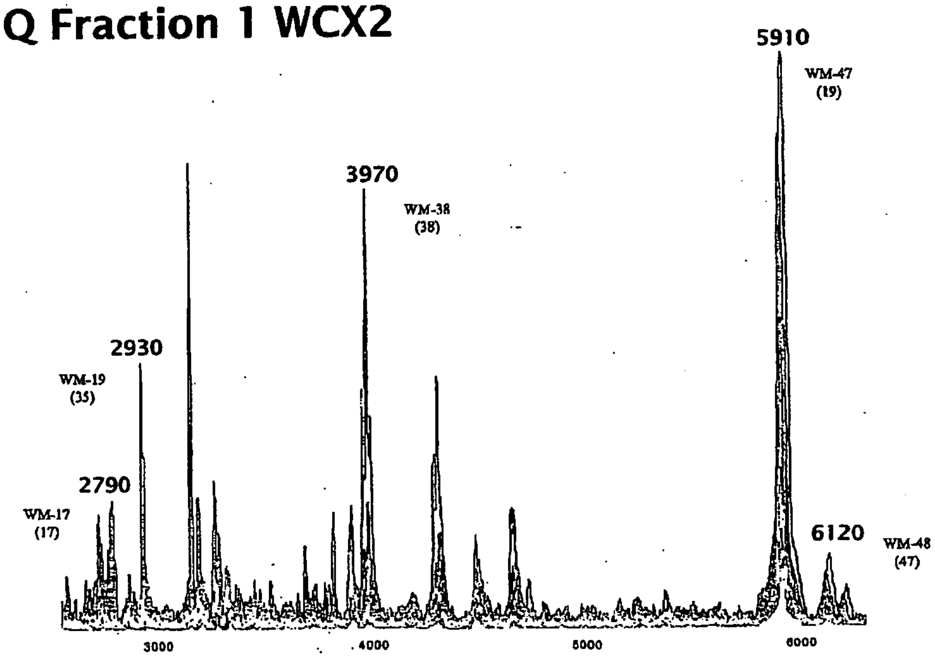


Figure 9

Protein Profile of Selected Samples Q Fraction 2 WCX2

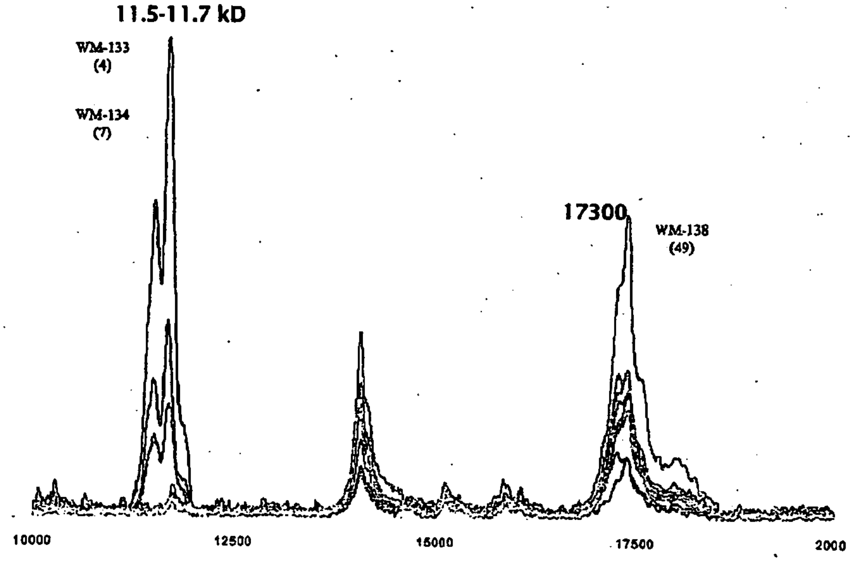


Figure 10

Protein Profile of Selected Samples Q Fraction 2 WCX2

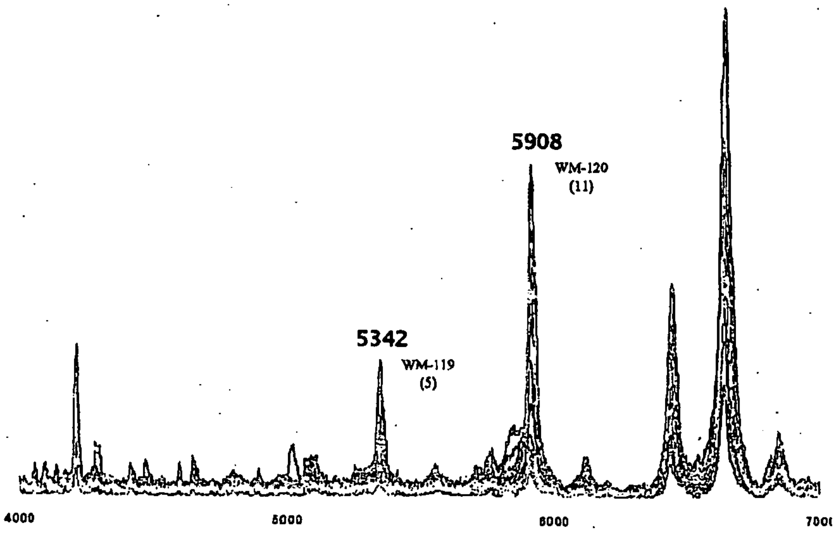


Figure 11
Protein Profile of Selected Samples
Q Fraction 4 WCX2

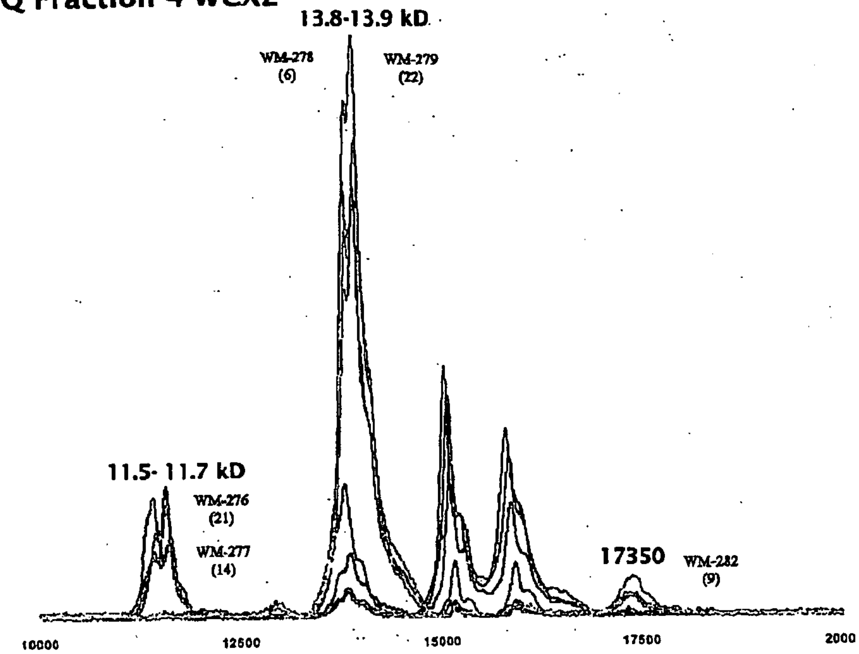


Figure 12
Protein Profile of Selected Samples
Q Fraction 4 WCX2

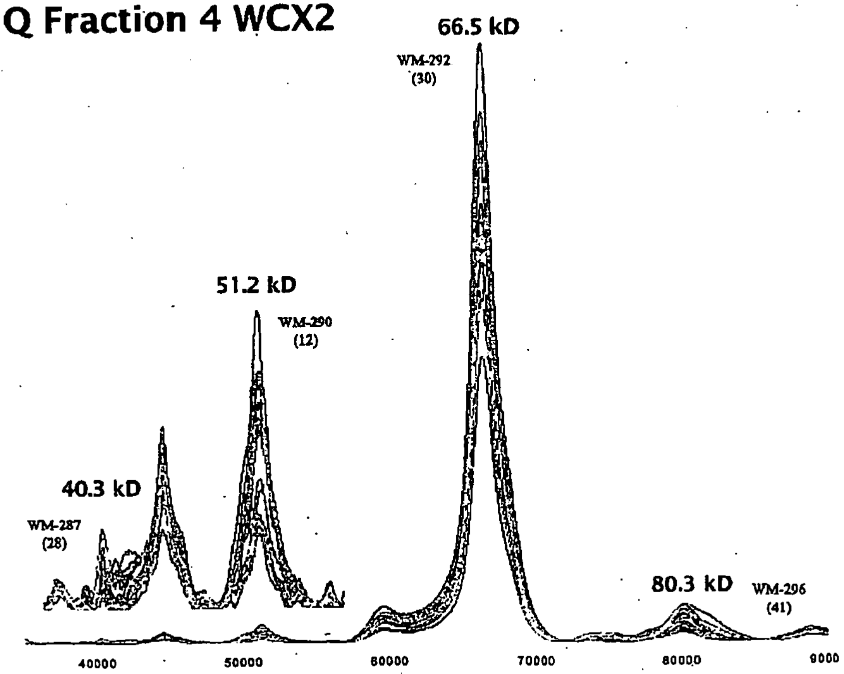


Figure 13
Protein Profile of Selected Samples
Q Fraction 5 WCX2

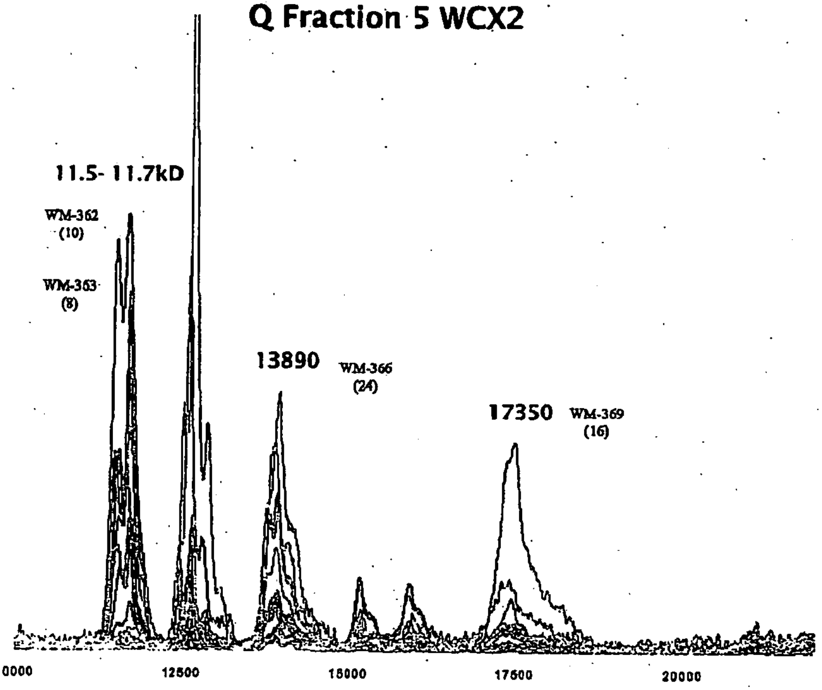


Figure 14
Protein Profile of Selected Samples
Q Fraction 5 WCX2

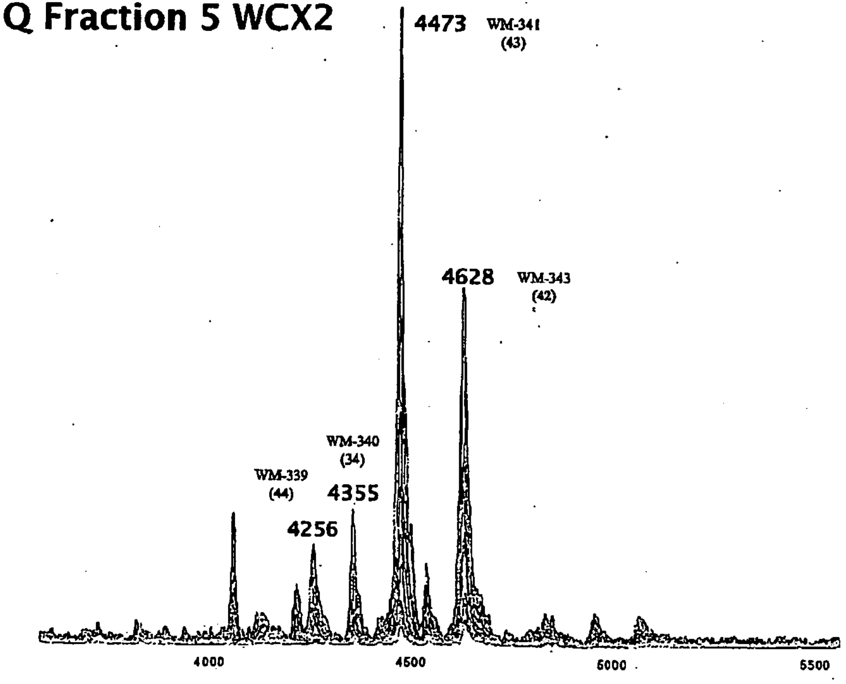


Figure 15

Protein Profile of Selected Samples

Q Fraction 6 WCX2

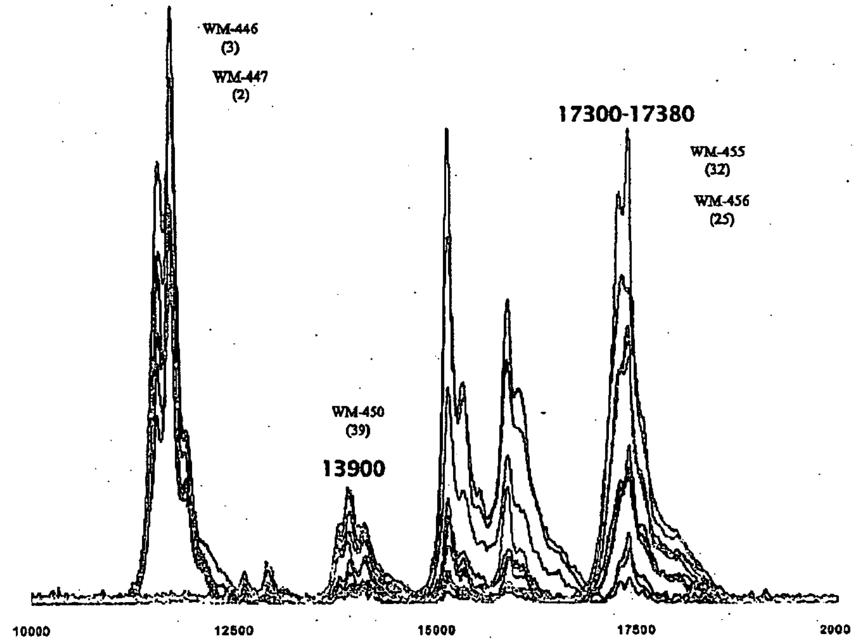


Figure 16

Protein Profile of Selected Samples Q Fraction 6 WCX2

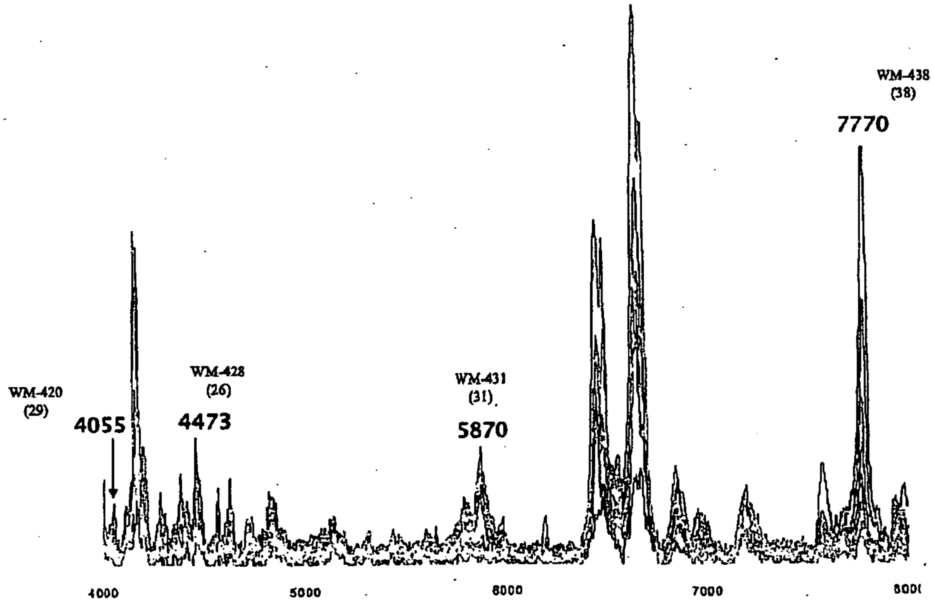


Figure 17

Protein Profile of Selected Samples Q Fraction 2 IMAC-Cu(II)

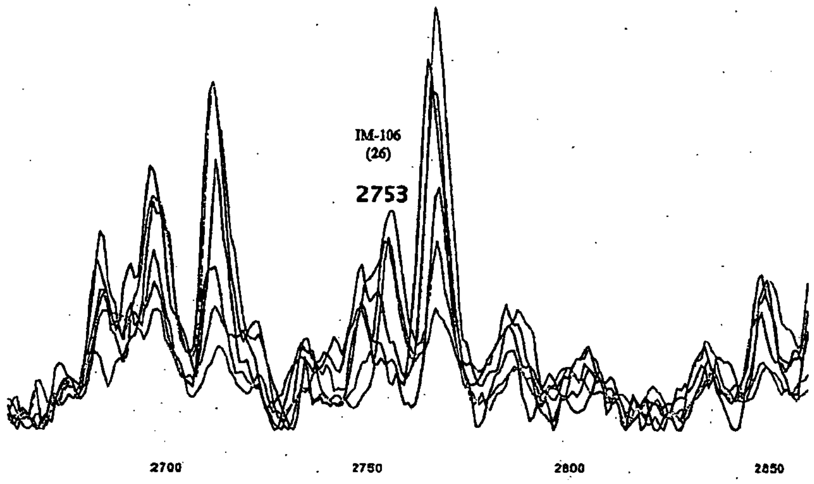


Figure 18

Protein Profile of Selected Samples Q Fraction 2 IMAC-Cu(II)

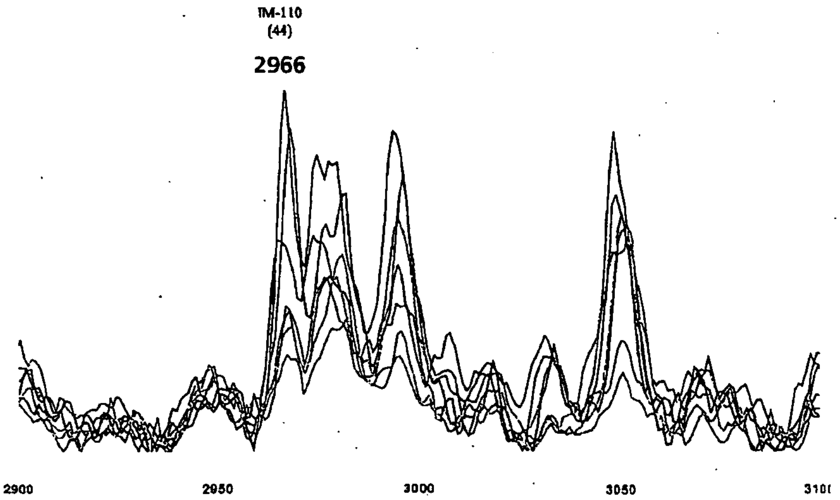


Figure 19
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)

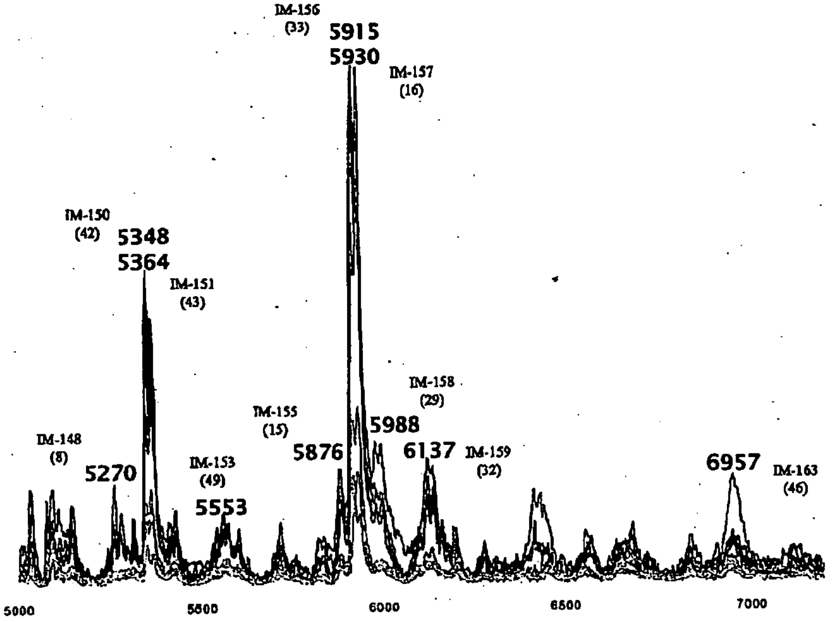


Figure 20
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)

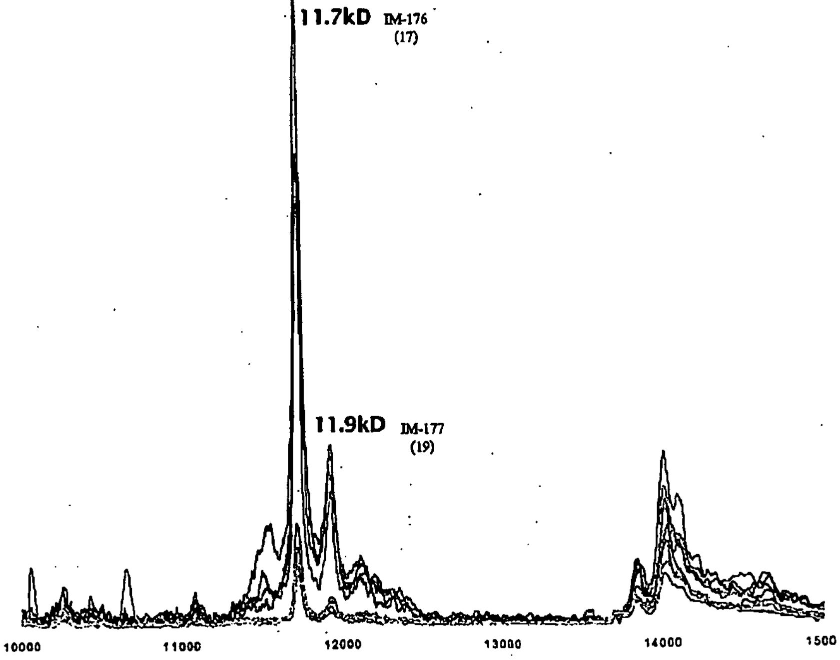


Figure 21

Protein Profile of Selected Samples Q Fraction 3 IMAC-Cu(II)

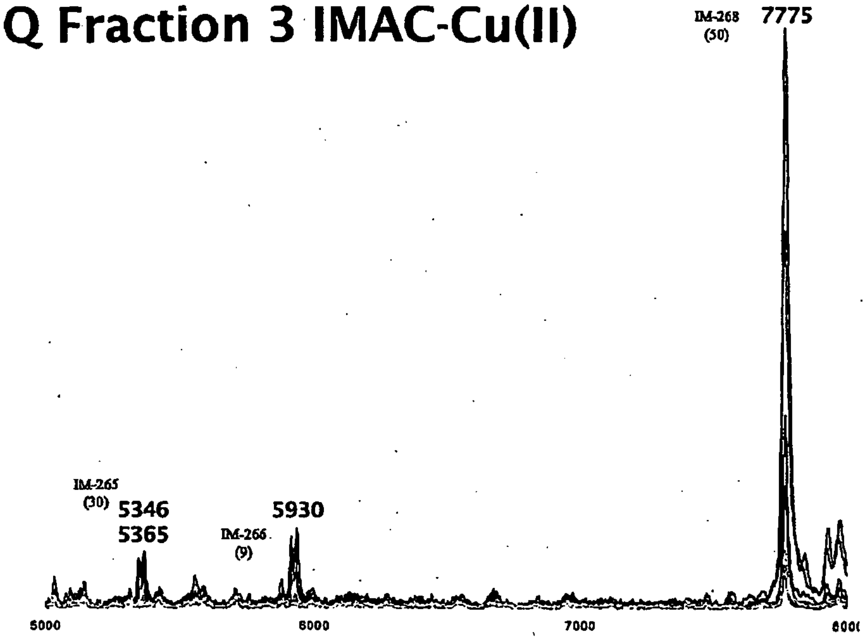


Figure 22

Protein Profile of Selected Samples Q Fraction 3 IMAC-Cu(II)

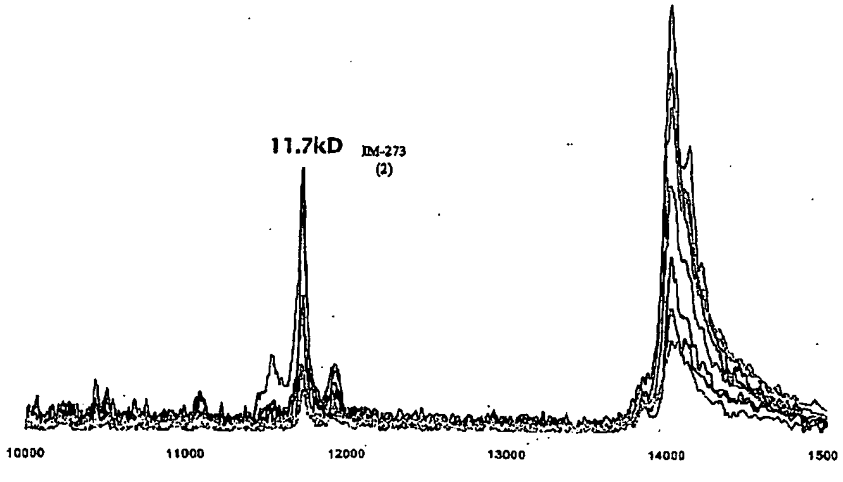


Figure 23

Protein Profile of Selected Samples
Q Fraction 5 IMAC-Cu(II)

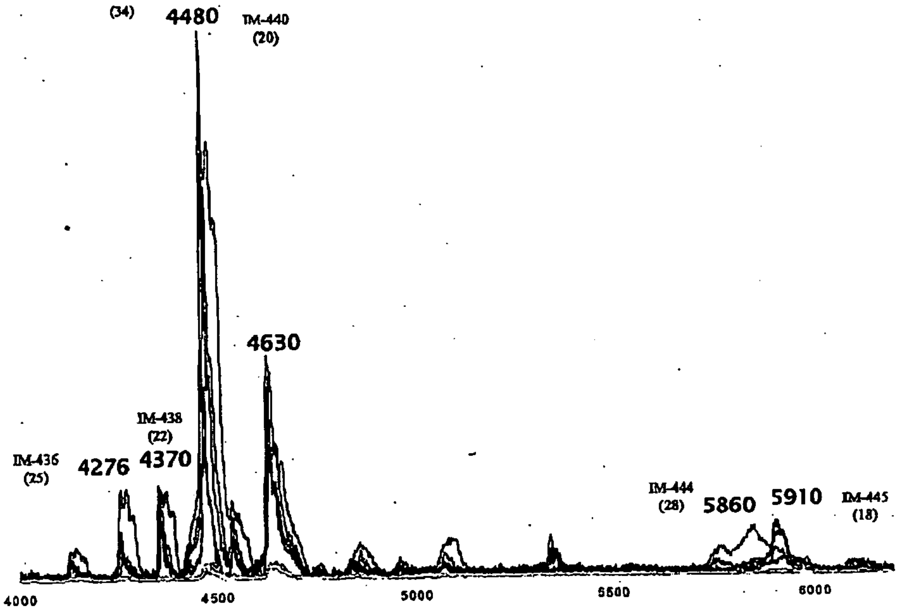


Figure 24

Protein Profile of Selected Samples
Q Fraction 5 IMAC-Cu(II)

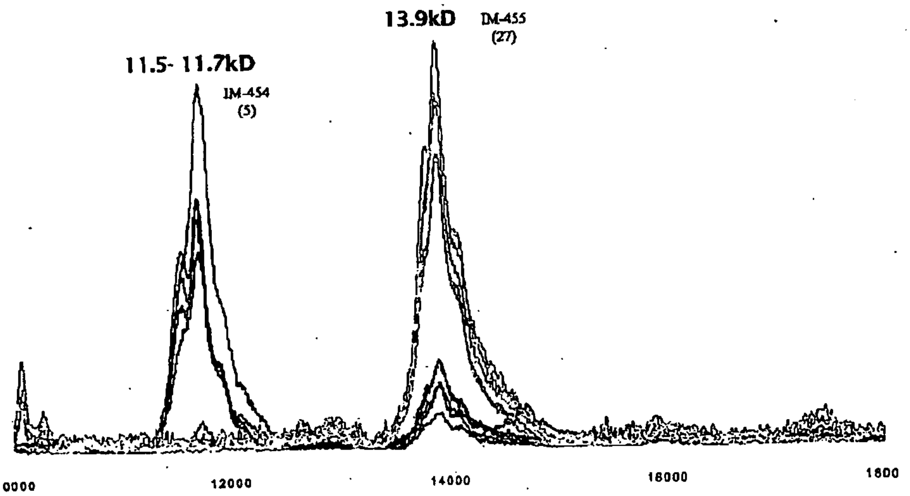


Figure 25

Protein Profile of Selected Samples
Q Fraction 5 IMAC-Cu(II)

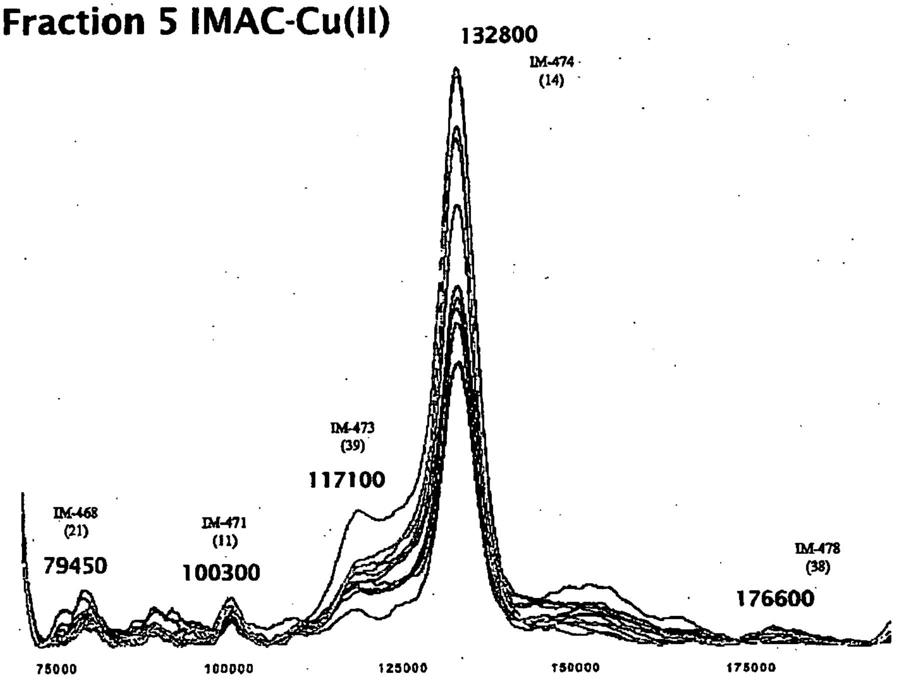


Figure 26

Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)

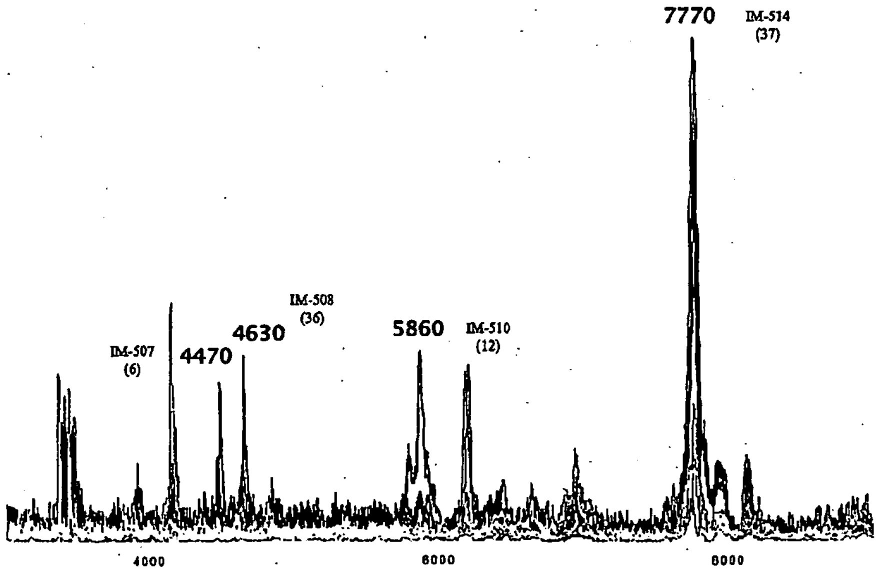


Figure 27
Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)

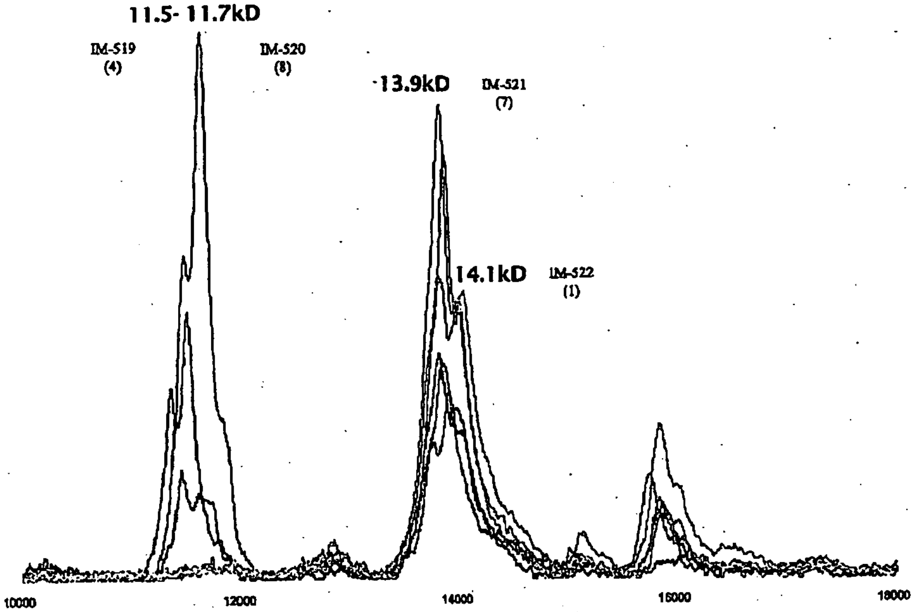
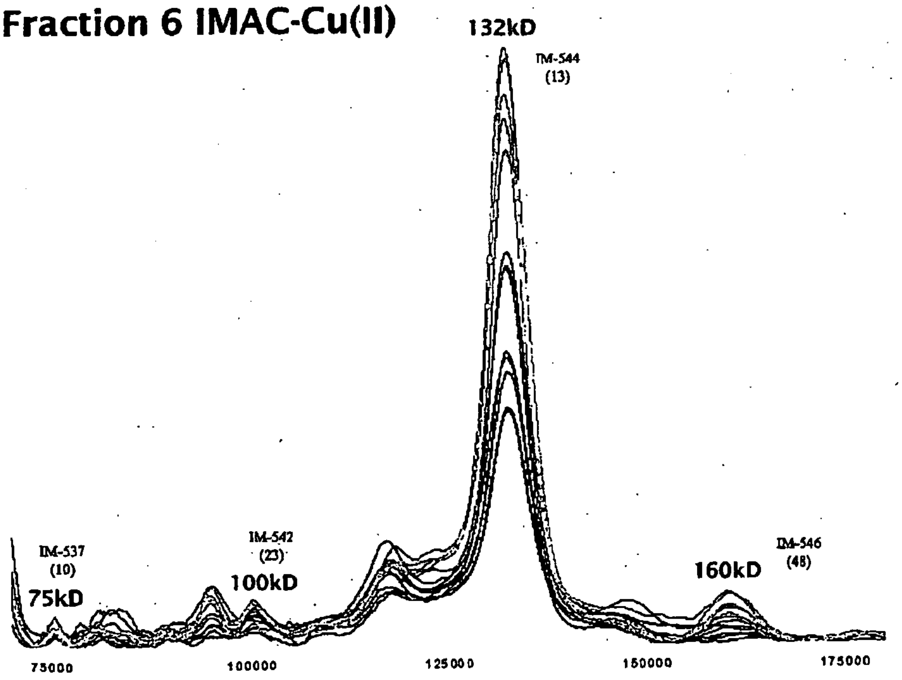


Figure 28
Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)



This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☐ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☐ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** ______________________________

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.